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Condition factor of *Amblypharyngodon chakaiensis* Babu and Nair in the Chakai boat channel (Trivandrum-Kerala-India)

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Abstract. Condition factor (K) and relative condition factor (K_n) of *Amblypharyngodon chakaiensis* Babu and Nair males and females respectively have been studied in relation to the various months of the year and length groups. No significant correlation exists between the proportion of the males in the population and value of condition factor (K). On the basis of K and K_n and the various length groups in respect of males and females are arranged into three classes and their incidence in the pre-spawning, spawning and post-spawning seasons were represented. In females K_n showed significant positive correlation with gonadosomatic index. The size at first maturity observed by direct observation of the gonads in females and males agrees with the inflexions on the curve depicting K and K_n with respect to length groups. The influence of feeding intensity on K does not seem to be quite apparent in *A. chakaiensis*. The steady fall in K in the males seems to be related to the decrease in the percentage occurrence of males of these length group in the sample.

Keywords. Condition factor; *Amblypharyngodon chakaiensis*; Chakai boat channel.

1. Introduction

The weight of the fish is said to vary with the cube of its length. Any deviation from this relationship has been reasoned as physiological changes in fish and is termed the 'condition factor' or 'ponderal index' (Hile 1936; Thompson 1943) which is calculated from the formula $K = 100W/L^3$ where K represents the condition factor, W the weight and L the length of the fish. By determining K it is possible to define the seasonal variation in the condition of the fish in relation to age and sex of the fish, and differences between the condition of the same species in different waters which might also serve as an index of the productivity of the water mass. The above formula is based on a comparison with ideal fish where the cubic relationship holds good.

Further modifications of the above relationships have been proposed. The K value will be affected if the fish does not obey the cube law in its length-weight relationship. Factors such as age, maturity, racial differences, food supply, degree of parasitization, environment and selection in sampling size may affect K indirectly through the exponent value. Instead, by using an empirical, $W = aL^n$ the length-weight relationship is calculated, and the factors affecting K could be eliminated (Le Cren 1951). The condition factor so calculated is called the relative condition factor (K_n) and is given by the formula $K_n = W/aL^n$. This may otherwise be denoted as $K_n = W/W$ where W represents the observed weight and W the calculated

weight of fish obtained by logarithmic formula. The difference, therefore, between K and K_n is that the latter measures the deviation of an individual fish from the average weight for length, while the former measures the deviation from a hypothetical ideal fish.

The present study deals with K in males and K_n in females in relation to season and length groups from samples collected from November 1978 to October 1979 from the Chakai boat channel, Trivandrum district, Kerala.

2. Material and methods

The K value for males and K_n value for females were calculated. Data on the length and weight of 158 males and 456 females ranging from 4.9 to 8.9 cm and from 5.4 to 9.38 cm respectively were calculated. The regular monthly samples were utilized for this purpose. Regression equations of length and weight were calculated separately for 158 males and 456 females. The equations were found to be

$$\log W = 2.9081 \log L - 1.6732 \quad (1)$$

$$\log W = 2.6861 \log L - 1.4220 \quad (2)$$

for males and females respectively (Babu 1981).

The K and K_n values were obtained for each male and female respectively and the arithmetic mean was calculated for each month and each length group taking the respective averages of the above values. The grand mean of K and K_n for monthly samples and various length groups for male and female *A. chakaiensis* respectively was also calculated for comparison.

3. Results

Values of K and K_n in respect of males and females of *A. chakaiensis* into various months and length groups are presented in tables 1 and 2 respectively.

3.1 The males

In November, December, January, May, September and October the K values remained below the grand mean. November, December, January and September registered very low K values, while in May and October the K values remained relatively near the grand mean. In all other months, the K values were higher and remained above the grand mean except in July when it remained at the grand mean. Though the proportion of males was low in the months with very low K values, no significant correlation could be drawn between the proportion of males and values of K since the proportion of males showed irregular fluctuations in the months with high K values (figure 1).

The K values of *A. chakaiensis* in respect of length groups reveal certain interesting features. The whole population throughout the period of observation was divided into ten length groups and designated as group I to X in order of length starting the lowest point from I. It will be seen that the values of K increase steadily from I to III and remain almost stable from III to VI (figure 2) and thereafter

Table 1. Means of condition factor, (K) and relative condition factor (K_n) of males and females of *A. chakaiensis* from November 1978 to October 1979.

Month	Mean K (male)	Mean K_n (female)
November 1978	0.11	0.95
December	0.08	0.97
January 1979	0.11	1.04
February	0.24	1.06
March	0.18	1.07
April	0.18	1.06
May	0.15	1.04
June	0.20	0.95
July	0.16	0.94
August	0.25	1.10
September	0.12	0.92
October	0.15	0.94
Grand mean	0.16	1.003

Table 2. Condition factor (K) and relative condition factor (K_n) of *A. chakaiensis* with respect to length groups.

Male		Female	
Length group (cm) standard length	K	Length group (cm) standard length	K_n
4.9-5.3	1.65	5.4-5.79	1.04
5.3-5.7	1.76	5.79-6.18	0.96
5.7-6.1	1.84	6.18-6.57	0.96
6.1-6.5	1.88	6.57-6.96	1.00
6.5-6.9	1.79	6.96-7.35	1.01
6.9-7.3	1.78	7.35-7.74	1.02
7.3-7.7	1.68	7.74-8.13	1.01
7.7-8.1	1.45	8.13-8.52	0.98
8.1-8.5	00*	8.52-8.91	1.04
8.5-8.9	1.15	8.91-9.30	1.03

*Samples were not available

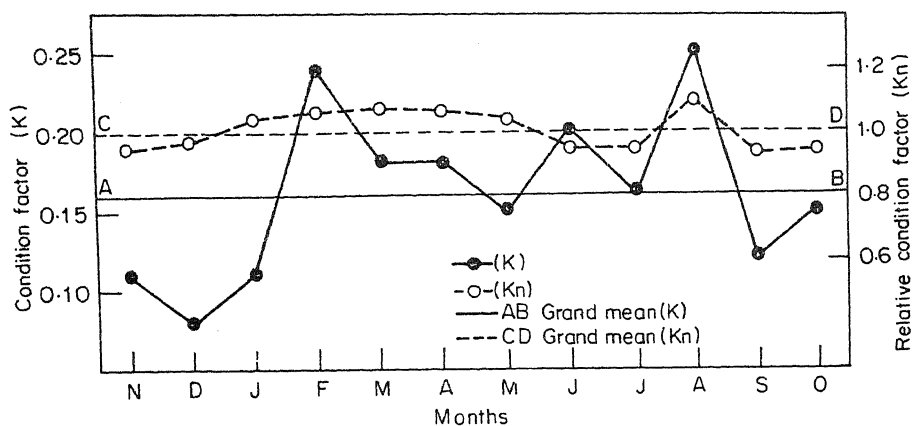


Figure 1. Condition factor and relative condition factor of *A. chakaiensis* in respect of months in the Chakai boat channel during 1978-79.

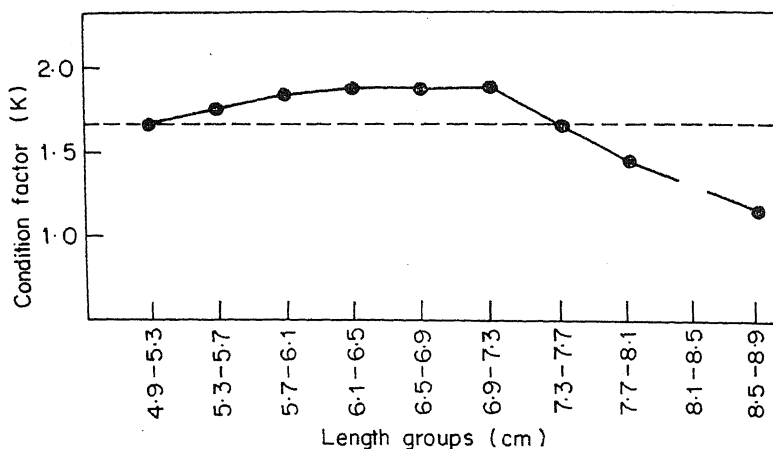


Figure 2. Condition factor of the male *A. chakaiensis* in relation to different length groups.

decrease sharply below the grand mean.

On the basis of the K values the ten groups were arranged into three classes and their incidence in the pre-spawning, spawning and post-spawning periods was determined (table 3). Class 1 comprised of length groups I to III, Class 2 of IV to VI and Class 3 of VII to X. Class 1 consisting of maturing and just matured individuals (since the size at first maturity of males was determined at 5.6 to 6.1 cm standard length) forms 43.41% for the whole period and 14.2% during the spawning period. More individuals of class 1 are seen in the pre-spawning period (59.25%) than post-spawning period (34.26%). Class 2 individuals form the largest percentage for the whole period (54.43) and spawning period (80.95%). Class 3 individuals are conspicuous by their absence in the pre-spawning period (those spawned in the previous breeding season probably do not survive up to the pre-spawning period) and scarce during the spawning and post-spawning periods.

Table 3. Percentage of male and female *A. chakaiensis* based on length groups and condition during the pre-spawning, spawning and post-spawning periods (from November 1978 to October 1979).

	Male			Female		
	I	II	III	I	II	III
Length Group (cm)	4.9-6.1	6.1-7.3	7.3-8.9	5.40-6.96	6.96-8.13	8.13-9.39
Range of condition factor (K)	1.63-1.84 (Mean 1.75)	1.79-1.88 (Mean 1.82)	1.15-1.65 (Mean 1.42)			
Range of relative condition factor (K_n)				0.96-1.04 (Mean 0.99)	1.01-1.02 (Mean 1.013)	0.96-1.04 (Mean 1.016)
% in the pre-spawning period	59.25	40.75	00	16.35	72.33	11.32
% in the spawning period	14.29	80.95	4.76	11.25	78.10	10.65
% in the post-spawning period	34.26	58.89	6.85	84.82	57.79	7.39
% for the whole period	42.41	54.42	3.16*	18.99	70.95	10.06

Pre-spawning period—February to May

Spawning period—June to September

Post-spawning period—October to January

3.2 The females

In the females the K_n values are below the grand mean in November, December, June, July, September and October and in other months above the grand mean (figure 1). The maximum K_n values was registered in August which coincides with the highest gonadosomatic index values (GSI). There is a positive correlation between K_n value and GSI (figure 3). The relationship between relative condition factor (Y) and gonadosomatic index (X) can be expressed as

$$Y = 0.0221X + 0.7773$$

and the correlation co-efficient (r) = 0.6402 which is significant at 5% level of significance.

The low K_n values during June and July can be attributed to low frequency spawnings in these months and that of September and October on account of the peak spawning in September. Low K_n values of November and December represent the post-spawning condition from which the female recovers gradually from January onwards.

The K_n values of the female in respect of the various length-groups exhibit certain interesting peculiarities. The population is arranged into ten length groups and these are designated as group I to X in order of length with the smallest form in group I. These groups are delineated under three classes. Class 1 consists of the first four length groups viz. 5.4 to 6.96 cm in standard length, class 2 consists of length

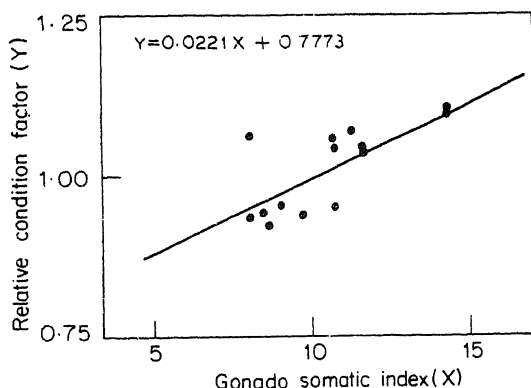


Figure 3. Gonadosomatic index-relative condition factor relationship in female *A. chakaiensis*.

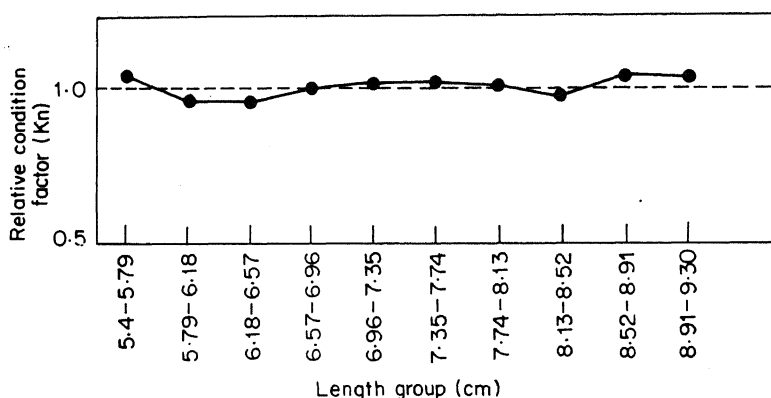
group V to VII (6.96 to 8.13 cm) and class 3 consists of VIII to X length groups (8.13 to 9.3 cm). The percentage occurrence for the whole period and for the pre-spawning, spawning and post-spawning periods was determined. Individuals of class 1 consist of maturing and recently matured ones (minimum length at first maturity determined by direct observation of the gonads was found to be between 6.5 and 6.9 cm in standard length) and they formed 18.99% for the whole period and 11.25% for the spawning period and these increase considerably in the post-spawning season to 34.82%. Class 2 formed 70.95% for the whole period and 78.10% for the spawning period. It is seen that class 2 occurs in its maximum frequency in the spawning period when the breeders are ready for spawning. A reduction in their percentage is observed in the post-spawning period. In class 3 the percentage occurrence of individuals is meagre when compared to the other two classes and formed 10.06% for the whole period and 10.65% for the spawning period. This class is consistent of low percentage occurrence in the pre-spawning, spawning and post-spawning periods indicating that the class is very lean owing to the effects of predation and ageing. The relative fecundity of this class is maximum when the relative fecundity and percentage occurrence during the spawning period are taken into consideration. Class 2 forms the dominant breeding group having the highest reproductive potential (table 4).

4. Discussion

Hart (1946) observed a point of inflexion on a curve showing the diminution of K with increase in length which indicated the length at which sexual maturity is attained. This feature has often been applied successfully in many fishes (Menon 1950; Pillay 1954; Sarojini 1957; Qayyum and Qasim 1964a, b, c; Kagwade 1969). But Bhatt (1968, 1970) observed that in the fish studied by him K did not give any indication of the size at first maturity. In male *A. chakaiensis* a gradual slope from the immature to the just matured length group is discernible from 4.9 to 6.1 cm (figure 2). In females the inflexion is conspicuous between 5.79 and 6.57 cm of standard length (figure 4). This is almost in agreement with the minimum size at first

Table 4. Relation of length group, relative condition factor (K_n) and relative fecundity of *A. chakaiensis* during November 1978 to October 1979.

Length group standard length (cm)	Range in K_n	Average relative fecundity (Number of mature eggs/g body weight)	Percentage occurrence of the length group in the whole period	Percentage occurrence of the length group in the breeding season	Reproductive potential as expressed as the product of columns 3 and 5
(1)	(2)	(3)	(4)	(5)	(6)
5.40-6.96	0.96-1.04 (Mean 0.99)	719	18.99	11.25	8088
6.96-8.13	1.01-1.02 (Mean 1.013)	957	70.95	78.10	74741
8.13-9.30	0.98-1.04 (Mean 1.016)	972	10.06	10.65	10352

**Figure 4.** Relative condition factor of the female *A. chakaiensis* in relation to different length groups.

maturity determined by the direct observation of gonads of the samples where it was seen that males and females attain maturity when they are 7.5 to 7.9 cm and 8.5 to 8.9 cm respectively in total length. When the standard length is reckoned for this purpose it will be 5.7 to 6.2 cm and 6.3 to 6.7 cm for males and females respectively. Thus the condition factor in *A. chakaiensis* readily indicates the size at first maturity as observed by Hart (1946). The incompleteness of the inflexion in the male is due to the non-availability of younger ones in the sample for length-weight studies. The presence of a less conspicuous inflexion in the K_n curve of the females of 8.13 to 8.52 cm length group (figure 4) needs further studies to arrive at definite conclusions.

Several factors have been pointed out by earlier investigators as influencing the condition of fish. Fluctuations in the gonad weight is the main factor which seems to regulate the condition factor (Le Cren 1951; Morrow 1951; Qayyum and Qasim 1964a, b, c). The other factor which seems to govern the rise and fall of K is the

feeding rate of fish (Qasim 1957; Bal and Jones 1960; Bhatt 1968). Bhatt (1970) observed in *Heteropneustes fossilis* and *Mystus seenghala* that the increase and decrease of K value gave no indication of the spawning season of these fish. He observed that the feeding rhythm was closely related to K than the cycle of gonad weight. He also observed that the feeding intensity overshadowed the effect of increase and decrease of gonad weight on the condition factor. The present observation of *A. chakaiensis* revealed that there is a conspicuous positive correlation between K_n values and gonadosomatic index (figure 3) and gonad volume in females (figure 5). In males also the relationship is distinct and thus is in agreement with the findings of Le Cren (1951); Morrow (1951); Qayyum and Qasim (1964a, b, c).

The influence of feeding intensity on the condition factor does not seem to be quite apparent in *A. chakaiensis*, yet a negative correlation is observed though not statistically significant (figure 6). Plant materials mostly algae and detritus dominate the food materials of *A. chakaiensis* and as such the weight of these items does not seem to be very conspicuous like the food items in carnivores such as *Heteropneustes fossilis* and *Mystus seenghala* (Bhatt 1968, 1970) where the feeding intensity has considerable effect on K values.

Nikolsky (1963) observed that sometimes a sharp reduction in the condition coefficient is seen associated with mass mortality. Qayyum and Qasim (1964a, b, c) observed fall in K values in larger fish of both sexes and this is possibly because of increasing metabolic strain on account of spawning in older age groups. Perhaps with increase in age, senility sets in and complete recovery which contributes towards reserve building and increase in weight gradually declines. Presumably this is the reason for their being poorer in condition than the younger breeders. The present study of *A. chakaiensis* reveals certain interesting features in the light of the above findings.

The steady fall in K and the sharp decrease in the percentage occurrence of males from 7.3 to 8.9 cm in standard length in samples (figure 2) indicate that the individuals in these length groups are being removed from the population by way of mortality. But in female *A. chakaiensis* the values of K_n do not show any sharp fall in the

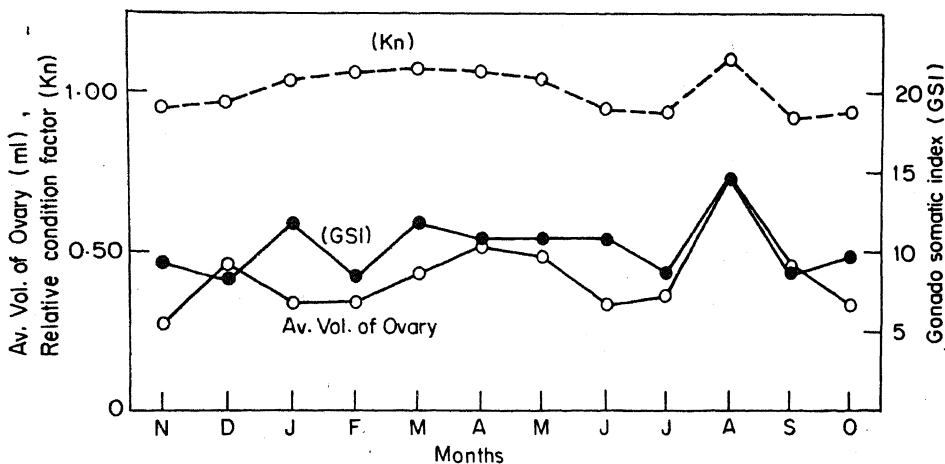


Figure 5. Relative condition factor, gonadosomatic index and average volume of the ovary in *A. chakaiensis*.

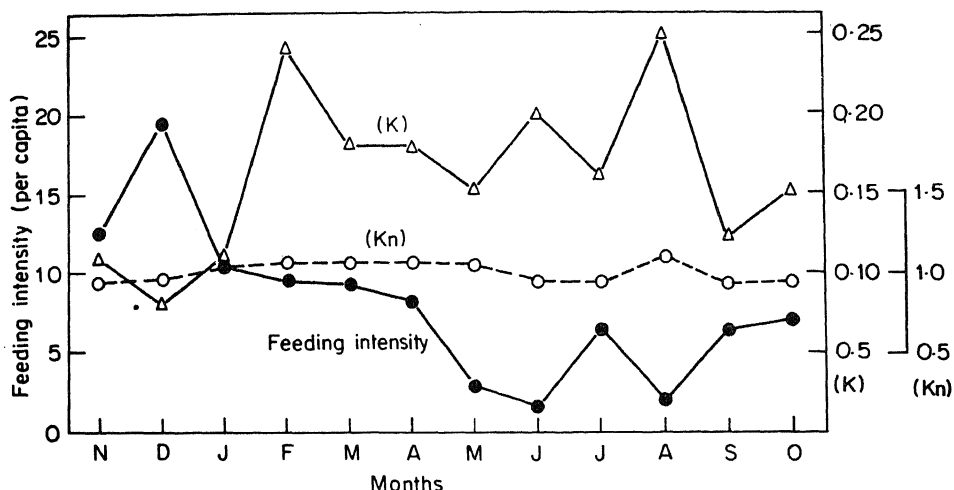


Figure 6. Relationship of condition factor and relative condition factor to per capita feeding intensity (combined) of *A. chakaiensis*.

higher length groups (figure 4) and the low percentage occurrence of these length groups viz. 8.13 to 9.3 cm in samples may probably be due to predation and fishing.

The apparent absence of specimens of male *A. chakaiensis* beyond 8.5 cm forces us to the irresistible conclusion that individuals beyond this size do not survive at all. It would also appear that in males, individuals beyond 7.3 to 7.7 cm in standard length represent the ageing class and they are gradually being removed from the population by mortality. The sharp fall in the *K* values suggest the advent of old age and consequent mortality as observed by Nikolsky (1963) and Qayyum and Qasim (1964a, b, c) and these are in agreement with the above findings.

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Gut spore composition and influence of fungal host on the rate of mortality and post-embryonic development in *Tiarothrips subramanii* (Ramk.)

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Abstract. Studies on the spore composition of the gut of the adult idolohipine sporophagous species *Tiarothrips subramanii* (Ramk.) indicated a greater preference for *Anthostomella consanguinea* as compared to others (viz.: *A. phoenicicola*, *A. sepelibilis*, *Pestalotia algeriensis*, *Melanographium citri*, *Phoma* sp., *Diplodia* sp., *Gnomonia* sp., *Stigmia palmivora*). The rates of development and mortality were higher when developing instars were reared on *A. phoenicicola* and *P. algeriensis* respectively.

Keywords. Gut spore composition; host fungi; fungal preference; mortality; post-embryonic development; *Tiarothrips subramanii*; *Borassus flabellifer*.

1. Introduction

Mycophagous Tubulifera involve essentially the mycetophagous phlaeothripines and sporophagous idolohipines. Ananthakrishnan and William James (1982) in a preliminary discussion on some of the idolohipine-fungal associations, refer to the complex of fungi (Ascomycetes, Coelomycetes, Basidiomycetes and Hyphomycetes) associated with *Borassus flabellifer* L. Information on the qualitative as well as quantitative analysis of the spore composition in the gut of Idolohipinae, appears important for a proper assessment of the spore preferences when a fungal complex is involved. Available information on these aspects being restricted to *Stictothrips fimbriata* (Anan.) fed on the fungi *Rhizopus* and *Pilobolus* (Joshi 1974) and to the biology of *Ecacanthothrips tibialis* (Viswanathan and Ananthakrishnan 1973), an attempt has been made to discuss the percentage composition of the gut spores in relation to the rates of mortality and post-embryonic development in *Tiarothrips subramanii*.

2. Material and methods

Different areas of the fungal-infested dried leaves of *B. flabellifer* were isolated, cultured in potato-dextrose-agar and oat-meal-agar media as indicated by Ananthakrishnan and William James (1982). The cultured fungi were mounted in lactophenol (phenol 20 gm, lactic acid 20 gm, glycerol 40 gm, and distilled water 20 ml) with cotton blue for identification. In order to prevent the germination of spores attached to the parts of the body of thrips, both adults and larvae were surface-sterilised using 0.01% mercuric chloride for 2-3 seconds and then washed in sterile water (distilled water sterilised in an autoclave at 1.1 kg/cm² for 30 min.) This ensured complete elimination of surface spores from contaminating the spore complex from the gut.

Spores removed from the gut were inoculated into the culture media, using an inoculation chamber. Three different methods were employed to culture such fungal spores. One set of spores was treated with 1% KOH, another with 1% HCl, and the third with sterile water. The treated spores were inoculated on the potato-dextrose-agar and oat-meal-agar media for culture.

The following fungi were isolated and identified from the specific areas of the leaf of *B. flabellifer*: (a) *Anthostomella phoenicicola* (Ascomycetes) from dead, dropped leaves. (b) *A. consanguinea* (Ascomycetes) from folds of leaves turning yellowish. (c) *Pestalotia algeriensis* (Coelomycetes) from the green leaves and (d) *Melanographium citri* (Hyphomycetes) from yellowing senescent leaves (figure 3-A).

To study the rates of mortality and post-embryonic development in *T. subramanii* on different fungi, freshly emerged first larvae were introduced into transparent plastic vials (4.5 × 3 cm) each containing the particular fungi-infested portions of the leaf of *B. flabellifer*. The percentage of larvae surviving to become adults indicated their preference for a particular fungus. Stock material was kept in a BOD incubator at $30 \pm 1^\circ\text{C}$ at 88% RH. Observations were made every day and the host leaf with the fungi replaced on alternate days. Spore counts from the dissected gut of adults and larvae of *T. subramanii* fed on different fungi were made, using a haemocytometer (figure 2).

Data on the percentage of postembryonic developmental rate and mortality rate were statistically examined and transformed to arc sine (percentage)^{1/2} values and subjected to analysis of variance (Snedecor and Cochran 1967).

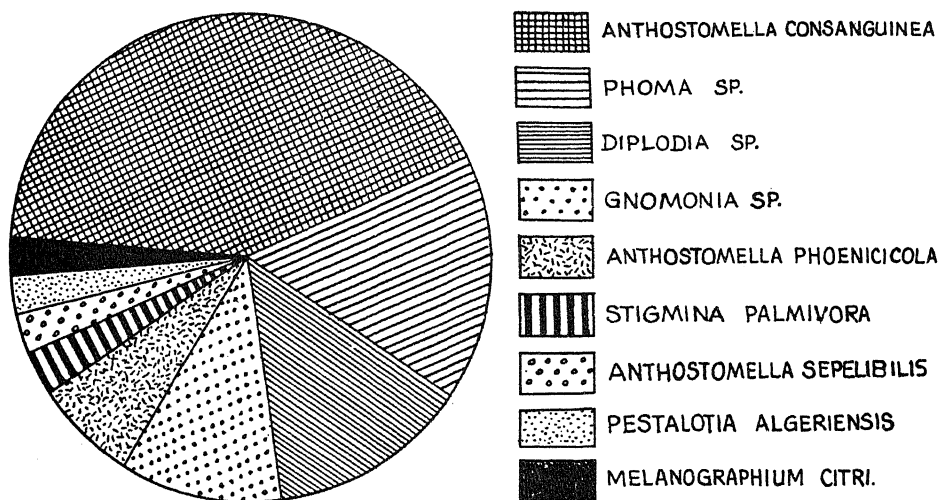


Figure 1. Distribution of different spores in the gut of adult *Tiarothrips subramanii*.

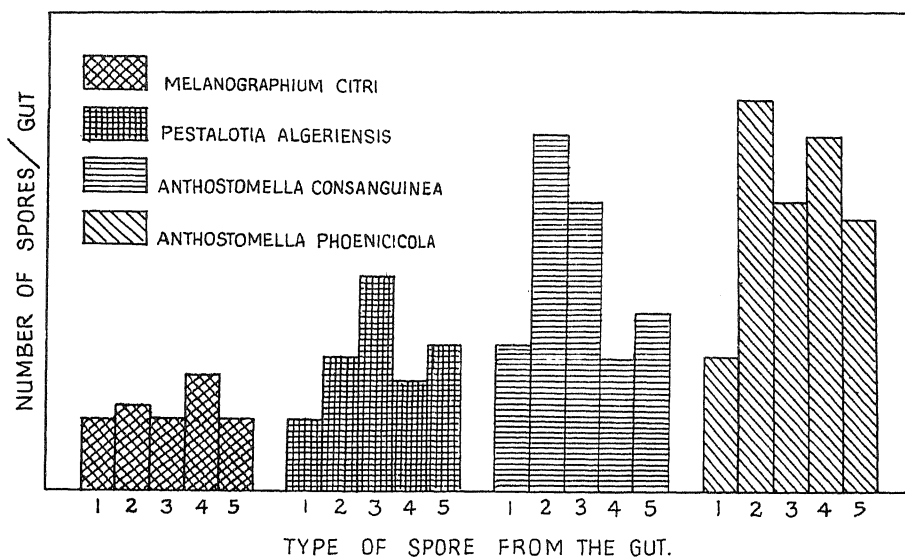


Figure 2. Gut spore composition of II larvae of *Tiarothrips subramanii* fed on different fungi (for five replicates).

Table 1. Gut spore composition of adult *Tiarothrips subramanii* (Mean of Six replicates)

Fungi	Percentage composition of spores in the gut*	
<i>A. consanguinea</i>	49.58 (44.79)	a
<i>Phoma</i> sp.	18.73 (24.75)	b
<i>Diplodia</i> sp.	12.18 (16.67)	c
<i>A. phoenicicola</i>	7.27 (14.93)	cd
<i>S. palmivora</i>	3.57 (6.17)	d
<i>A. sepelibilis</i>	2.47 (5.26)	d
<i>P. algeriensis</i>	2.43 (6.33)	d
<i>Gnomonia</i> sp.	1.05 (3.38)	d
<i>M. citri</i>	0.33 (1.36)	d

* Figures in parenthesis are arc sine (percent)^{1/2} values.
Means followed by a common letter are not significant
at 1% level.

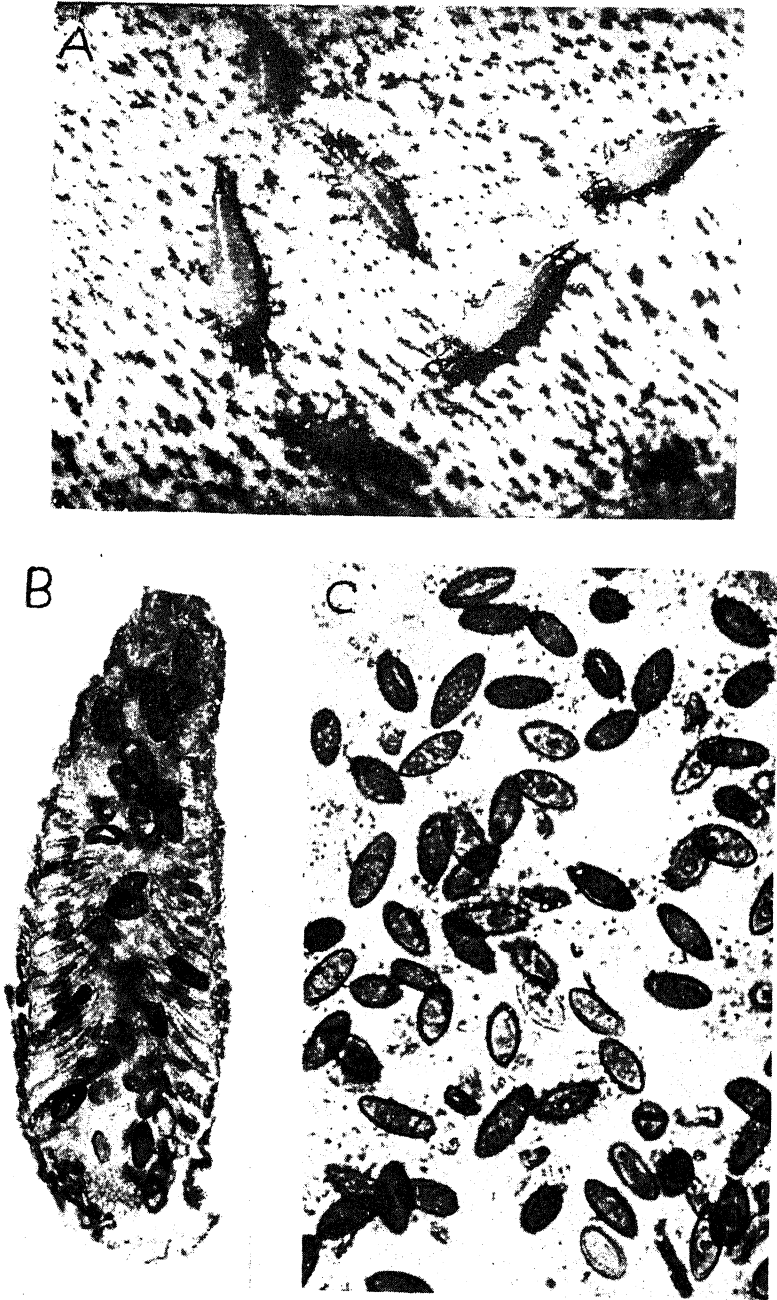


Figure 3. A. *Tiarothrips subramanii* larvae feeding on *Melanographium citri* B. C.S. of *T. subramanii* foregut showing the spores of *Anthostomella consanguinea* C. Spores of *Anthostomella consanguinea* from the gut of *T. subramanii*.

3. Observations

3.1. Gut spore composition

Gut spore analysis of the adult *T. subramanii* fed on mixed fungi, revealed significant percentage of *Anthostomella consanguinea* (49.58), *Phoma* sp. (18.73), and *Diplodia* sp. (12.18), whereas the other spores viz., *A. phoenicicola*, *Stigmina palmivora*; *A. sepelibilis*, *Pestalotia algeriensis*, *Gnomonia* sp., *Melanographium citri* occurred in very insignificant percentage (table 1, figure 1).

3.2. Duration of different instars fed on various fungi

Duration of the larval and pupal periods varied with each fungal host. The first larval stage lasted for 1-2 days, 2 days, 2-3 days, and 1-2 days when fed on the spores of *A. consanguinea*, *P. algeriensis*, *M. citri* and *A. phoenicicola* respectively. Duration of the second larval period varied from 3-4 days when reared on *M. citri*, *A. phoenicicola*, *A. consanguinea* and 6-7 days in *P. algeriensis*. 20% of second stage larvae did not moult further when cultured on *P. algeriensis*, while 26% and 28% of larvae failed to moult further when reared on *A. consanguinea* and *M. citri* respectively (table 2).

Table 2. Fungal preference and its relative effect on larval mortality and post-embryonic development in *Tiarothrips subramanii* (Ramk.) (mean of five replications).

	Host fungi from dried leaves of <i>B. flabellifer</i>			
	<i>A. phoenicicola</i>	<i>A. consanguinea</i>	<i>M. citri</i>	<i>P. algeriensis</i>
Development rate (%)				
L ₁	96 (84.7)	92 (82.2)	100 (90)	100 (90)
L ₂	70 (60)	28 (28.6)	12 (13.1)	8 (7.9)
PP	100 (90)	98.8 (69.2)	40 (36)	20 (18)
P ₁	100 (90)	64 (54.2)	40 (36)	20 (18)
P ₂	100 (90)	80 (72)	40 (36)	10 (9)
Mortality rate (%)				
L ₁	4 (5.3)	8 (7.5)	0 (0)	0 (0)
L ₂	17 (18.9)	48 (43.5)	60 (53.8)	72 (61.4)
PP	0 (0)	1.2 (2.8)	0 (0)	0 (0)
P ₁	0 (0)	14.4 (16.1)	0 (0)	0 (0)
P ₂	0 (0)	0 (0)	0 (0)	10 (9)
Larvae that failed to grow and moult further				
L ₁	0 (0)	0 (0)	0 (0)	0 (0)
L ₂	13 (16.6)	26 (24.6)	28 (28.9)	20.8 (20.8)

L₁ first larva; L₂ second larva; PP prepupa; P₁ first pupa; P₂ second pupa; Data significant at 1% level. Figures in parenthesis are arc sine (percent)^{1/2} values.

CD at 1% level

Between fungal spores: 6.7; Between larval stages: 7.04; Between larval mortality: 26.8; Fungus × larval mortality: 14.9; Larval stages × larval mortality: 19.5; Fungus × thrips: Not significant; Fungus × Thrips × larval mortality: Not significant.

3.3 Rates of mortality on various fungi

Larvae reared on *A. phoenicicola* showed 4% mortality in the first larval stage and 17% in the second. The overall mortality during the entire developmental period was 32%. Those reared on *A. consanguinea* showed 8%, 48%, 1.2% and 14.4% mortality during first, second, prepupal and first pupal stages respectively, and an overall mortality of 58% during the entire period of development. Larvae cultured on *M. citri* indicated 60% mortality in the second larval stage with no mortality during the rest of the stages. In *P. algeriensis* mortality rates of 72% and 10% were observed in second larval and second pupal stages (table 2).

3.4 Rate of development on various fungi

Thrips cultured on different fungi indicated not only their relative fungal preference during development, but also the influence of the concerned fungi on the rates of development. Post-embryonic developmental period was of the order of 7-10 days when reared on both *A. phoenicicola* and *A. consanguinea*. A comparatively longer duration of 8-11 days and 11-12 days was observed when reared on *M. citri* and *P. algeriensis* respectively. The percentage of adult emergence also varied considerably when reared on the above-mentioned fungi, 56% of larvae developing into adults when fed on *A. phoenicicola*, 16% on *A. consanguinea*, 12% on *M. citri* and 4% on *P. algeriensis*. As already mentioned, mortality rate was considerably less when reared on *A. phoenicicola*, slightly higher on *A. consanguinea* and very much so on *M. citri* and *P. algeriensis*. The rate of development and percentage of adult emergence appeared high when reared on *A. phoenicicola*, low in *A. consanguinea* and very much lower on *P. algeriensis* and *M. citri*.

4. Discussion

Spore analysis of the gut of the adults fed on a complex of fungi infesting the leaves of *B. flabellifer* revealed only the presence of large numbers of *A. consanguinea* (table 1, figure 1) the other types of spores occurring in negligible numbers. The gut spore analysis of larvae fed on different fungi such as *A. phoenicicola*, *A. consanguinea*, *P. algeriensis* and *M. citri* also showed that *A. phoenicicola* was preferred by the first and second larval stages (figure 2), when cultured on these fungal species at $30 \pm 1^\circ\text{C}$, 88% RH, the duration of first instar larvae to adult was 7-10, 8-11, and 11-12 days respectively. The preference of *A. phoenicicola* by the developing larvae and of *A. consanguinea* by the adult *T. subramanii* therefore appears to be of interest. Such observations on the relative preference for particular fungi as well as on the variations involved in the rate of development and in the percentage of adult emergence have also been made on the fungal mite *Galumna flabellifera* Hammer (Reddy *et al* 1978).

Spore analysis of the gut of larvae fed on *M. citri* revealed the presence of young conidia of *M. citri* in different stages of development. Larvae when fed on other fungi such as *A. consanguinea*, *A. phoenicicola*, and *P. algeriensis* showed only mature spores. *M. citri* being a hyphomycete, the conidia in various stages of development were found on the leaf surface, whereas in the other fungi, the early developmental stages were located inside the fruiting body of the fungi and only mature spores were available on the leaf surface.

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Response of some freshwater micrometazoans to DDT

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Abstract. The response of a few members of freshwater micrometazoans to DDT have been ascertained by conducting laboratory and field bioassay experiments. The organisms tested in laboratory bioassays, have been found to respond at low concentrations with LC50 ranging between 10 and 49 ppb. *Cyclops* sp. and *Asplanchna brightwelli* respond to higher concentrations of DDT with LC50 as high as 515 ppb and 2218 ppb respectively. LC50 for the larvae of *Culex fatigans* has been found to be 180 ppb. In the field test of 70 ppb of DDT was applied and repeated after 23 days. The response of organisms is similar in both the treatments. The population of *C. cornuta* and *M. brachiata* reappeared after 22 days of the treatment whereas, *D. excisum* and *N. kamakhiae* failed to reappear. Analysis of the bottom soil of the treated tank revealed the presence of DDD, DDE and DDT. Some secondary effects of the DDT treatment have also been observed and discussed.

Keywords. Freshwater micrometazoans; bioassay; DDT; mortality; aquatic weeds.

1. Introduction

Insecticide pollution is a serious problem across the world especially in developing countries where it is used indiscriminately in crop protection programmes. During monsoon these toxic chemicals easily find their way along with water into ponds, lakes and rivers causing damage to biotic communities.

Literature perusal reveals that there have been excellent studies on toxicity of pesticides in fish but similar work on freshwater micrometazoans is limited (Anderson 1945; Ruber 1963, 1965; Sanders and Cope 1966, 1968; Patterson and Windeguth 1964; Ruber and Basker 1968; Hurlbert *et al* 1970, 1972; Khudairi and Ruber 1973; Hurlbert 1975; Ali and Mulla 1978). The present study aims at evaluating the toxicity of DDT on a few members of freshwater microfauna which are important in the aquatic food chain.

2. Material and methods

2.1 Laboratory bioassay experiments

Test organisms, viz., *Ceriodaphnia cornuta*, *Neodiaptomus kamakhiae*, *Moina brachiata*, *Diphanosoma excisum* and *Cyclops* sp. were collected from Jyotisar, a freshwater tank in Kurukshetra. Laboratory cultures of *Ceriodaphnia reticulata* and *Asplanchna brightwelli* raised by employing the methods of Dewey and Parker (1964) and Singh and Dattagupta (1981), respectively, have been used in the bioassay. To obtain mosquito larvae, yeast contaminated tap water was left open in glass jars during the night. Egg rafts laid in these glass jars by wild *Culex fatigans* were collected next day. Each egg raft was kept in a separate container and allowed

to hatch in tap water. The newly hatched larvae were given yeast suspension as food till they reached their 4th instar stages.

The bioassay experiments were performed on the animals of uniform age in *A. brightwelli*, *C. cornuta*, *C. reticulata*, *D. excisum* and *M. brachiata* since these organisms reproduce young ones parthenogenetically. One day prior to testing, the brooded forms of the aforesaid animals were placed in the classified petri dishes, and the young ones which appeared on the following day were considered to be not more than one day old and were used in the bioassay. With regard to *Neodiptomus kamakhiae* and *Cyclops* sp. the animals of uniform size, and for the larvae of *Culex fatigans* only 4th instar stages were put to laboratory bioassay treatments.

One percent stock solution of DDT was prepared in acetone. The insecticide was further diluted from this stock solution. The coarsely filtered water of the Jyotisar tank was used as dilution medium for experiments using the organisms collected from this tank. The rotifer media (Singh and Dattagupta 1981) were used for experiments using *A. brightwelli*. Aged tap water, kept for a week in a closed container, was taken as diluent for experiments using *C. reticulata* and the larvae of *Culex fatigans*.

The toxicity experiments on *N. kamakhiae*, *C. cornuta*, *C. reticulata*, *M. brachiata*, *D. excisum*, *A. brightwelli* and *Cyclops* sp. were conducted in cavity blocks of 15 ml capacity containing 10 ml of test concentration. The larvae of *Culex fatigans* were treated in glass beakers of 250 ml capacity containing 200 ml of test concentration. Ten micrometazoans were used in each assay treatment and 3 replications of each treatment were maintained. The experiments employing each species were repeated thrice. Controls were run for each of these experiments. Acetone was added to the control in an amount equal to the largest aliquot of stock solution used in the test concentration.

The mortality was recorded after 24 hr treatment in all experiments except those performed on *A. brightwelli*. The mortality of *A. brightwelli* was observed after 12 hr treatment because a longer exposure period would cover over 20% of the average life span of this species. The toxicity of the insecticide was calculated as LC50 (concentration lethal to 50% of the test organisms) using the standard methods (Busvine 1971).

2.2 Field bioassay experiment

Two small tanks ($5 \times 5 \times 2$ ft) were constructed to study the DDT effect on the population of different micrometazoans in their natural habitat. The natural population of different micrometazoans was obtained from their resting eggs present in each experimental tank. The study began in August 1979 and terminated in November 1979 and during this period one of the experimental tanks was given two DDT treatments at the same concentration of 70 ppb on 11 September and 4 October, respectively. The other tank was left untreated to run as a control. Microfauna samples were collected from each experimental tank. The first sample was taken on the third day after the treatment; thereafter the samples were drawn every 10 days. For a sample, water (20 litres) taken from different spots of the tank was filtered through Hand plankton net. The concentrated mass of microfauna thus obtained was estimated for the different organisms. The total organisms present in one litre were estimated for each sample. Temperature, pH, dissolved O_2 of water were also measured at the time of each collection. After the experiment, the soil

samples taken from the top 3 cm bottom sediments of each experimental tank were examined for various residues of DDT using gas liquid chromatograph, Packard A7400 equipped with electron capture detector with tritium foil.

3. Results and discussion

The concentration tested and the percentage of mortality were converted to logs and probits respectively and calculated to get regression equation by the least square method for each test species (figures 1 and 2). Table 1 represents the LC50 value of the different organisms tested in the laboratory. It is evident from these values that *N. kamakhiae* is more sensitive to DDT than the other organisms tested. Further, the susceptibility of the other micrometazoans is in the order as *C. cornuta*, *D. excisum*, *C. reticulata*, *M. brachiata*, larvae of *Culex fatigans*, *Cyclops* sp. and *A. brightwelli*; the latter being least susceptible. These test organisms could be grouped into two broad categories based on their tolerance to DDT concentrations. *N. kamakhiae*, *C. cornuta*, *D. excisum*, *C. reticulata*, *M. brachiata* respond to low concentrations with LC50 ranging between 10 and 49 ppb. Compared with these microcrustaceans, *Cyclops* sp. and *A. brightwelli* have been found to respond to much higher concentration with LC50 as high as 515 ppb and 2218 ppb, respectively.

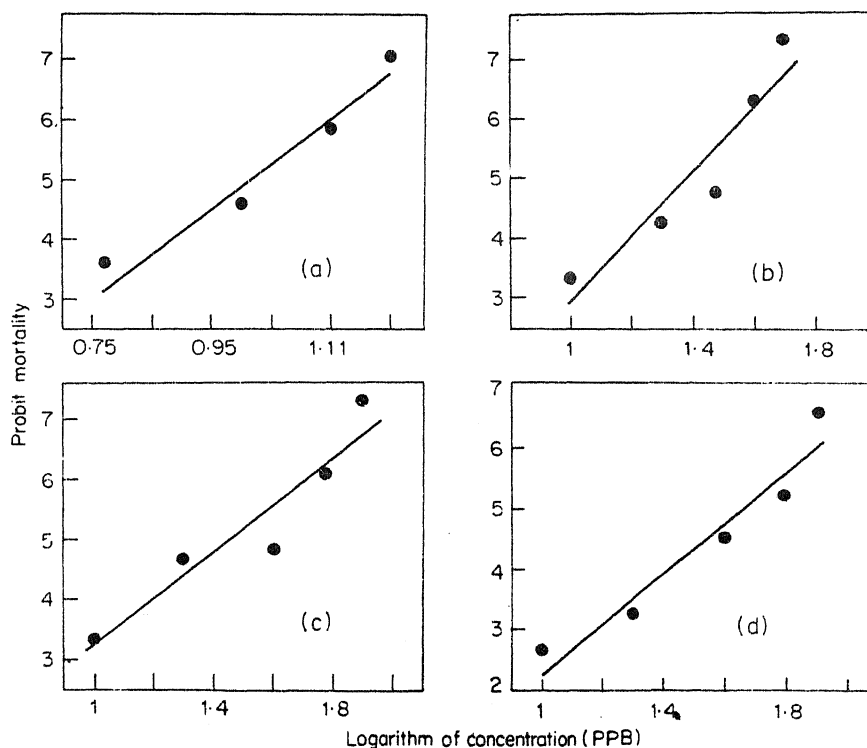


Figure 1. Response of some freshwater micrometazoans to DDT. **a** = *Neodiaptomus kamakhiae*, **b** = *Ceriodaphnia cornuta*, **c** = *Diphanosoma excisum*, **d** = *Ceriodaphnia reticulata*

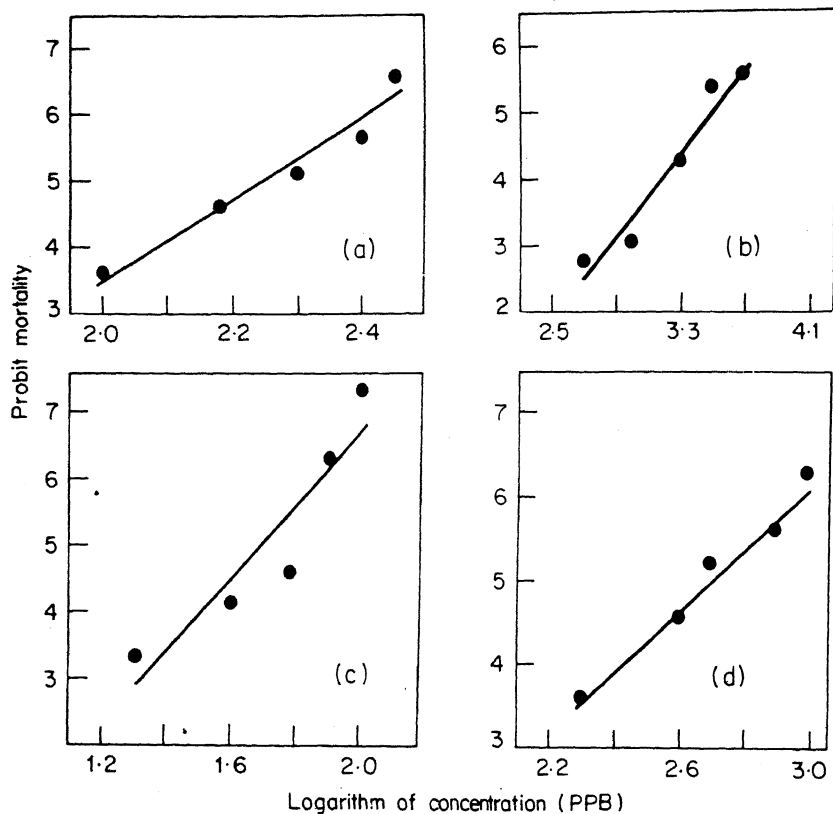


Figure 2. Response of some freshwater micrometazoans to DDT. **a** = larvae of *Culex fatigans*, **b** = *Asplanchna brightwelli*, **c** = *Moina brachiata*, **d** = *Cyclops sp.*

Table 1. LC50 values of different organisms.

Organisms	LC50 (ppb)
<i>Neodiaptomus kamakhiae</i>	10
<i>Ceriodaphnia cornuta</i>	23
<i>Diphanosoma excisum</i>	28
<i>Ceriodaphnia reticulata</i>	44
<i>Moina brachiata</i>	49
Larvae of <i>Culex fatigans</i>	180
<i>Cyclops sp.</i>	515
* <i>Asplanchna brightwelli</i>	2218

*Exposed for 12 hr, others for 24 hr

Although it is not possible to attribute any reason to the tolerance, both *A. brightwelli* and *Cyclops* sp. are raptorial feeders (Hutchinson 1967). Compared with the latter organisms *N. kamakhiae*, *C. cornuta*, *C. reticulata*, *D. excisum*, *M. brachiata* are readily susceptible to DDT and interestingly enough these organisms are filter feeders (Hutchinson 1967). Filter feeding zooplankters have been known to ingest more pesticide than other forms of freshwater micrometazoans (Hurlbert 1975). Ruber (1963) reported that the rotifer, *Philodina* sp. was unaffected at a concentration of 500 ppb of DDT exposed for 193 hr. Further, Hurlbert *et al* (1972) reported an increase in population and increase in sexual forms of *A. brightwelli* in ponds treated with Dursban, an organophosphate. The LC50 of 180 ppb for the larvae of *Culex fatigans* as has been found in the present study is considerably higher than what has been reported by WHO (1968) for larvae of *Culex fatigans*. The larvae for the present experiment have been obtained from eggs laid by wild *Culex fatigans*. Since, the university campus is regularly sprayed with chlorinated hydrocarbons as a measure of malaria control, it is possible that tolerance level of these mosquito larvae have reached a higher level consequent upon their exposure to insecticide residues. Table 2 shows the toxicity of DDT to a variety of freshwater micrometazoans reported by several authors. But these authors worked on species of aquatic animals which are different from the present ones. Also there could be a difference in response owing to the intrinsic heterogeneity of the test organisms.

Table 2. Showing the toxicity of DDT to a variety of freshwater micrometazoans.

Organism	Toxicity measurements (in ppb)			References
	Acute Range (0 to 100% mortality) (24 hr)	LC50 (24 hr)	EC50** (48 hr)	
<i>Daphnia pulex</i>	—	—	0.36	Sanders and Cope (1966)
<i>Simocephalus serrulatus</i>	—	—	2.50	—do—
<i>Daphnia magna</i>	—	—	1.40	Boyd (1957)
<i>Microcyclops bicolor</i>	30-250	—	—	Ruber (1963)
<i>Ceriodaphnia quadrangula</i>	6-120	—	—	—do—
<i>Cypridopsis vidua</i>	250-1000	—	—	—do—
<i>Cyclops apocyclops</i>	10-500	—	—	Ruber and Basker (1968)
<i>Diaptomus</i> sp.	1-100*	—	—	—do—
Larvae of <i>Culex fatigans</i>	—	70	—	WHO (1968)
Larvae of <i>Anopheles albimanus</i>	—	10	—	Georghiou (1972)
Stonefly naid (<i>Pteronarcys californica</i>)	—	41	—	Sanders and Cope (1968)

*Acute range for 40 to 100% mortality

**50% immobilization of the organisms

In field bioassay experiment, *C. cornuta* and *M. brachiata* were found completely absent within 2 days of the treatment. However, these organisms reappeared in the samples which were collected after 22 days of the treatment (figure 3). These reappeared forms apparently were hatched from their respective dormant eggs. The treatment of the same concentration was repeated and the response of the organisms has been similar to that after the first treatment. Therefore, the young ones emerging from the dormant eggs seem to have the same level of tolerance as that of their previous generations. Shiff and Bridget (1961), Hurlbert *et al* (1970, 1972) Didia *et al* (1975) and Ali and Mulla (1978) found similar micrometazoans reappear at an interval of 1 to 3 weeks after they were eliminated partially or totally by insecticide treatments in the field.

N. kamakhiae and *D. excisum* were completely absent after 2 days of the first DDT treatment (figure 4). However, they were present throughout the study period in the control tank. Miura and Takahashi (1976) also reported similar failure of some cladocerans to reappear after the water was treated with 0.03 ppm of SD 47775. In a similar situation, *Diaptomus pallidus* did not develop in the experimental

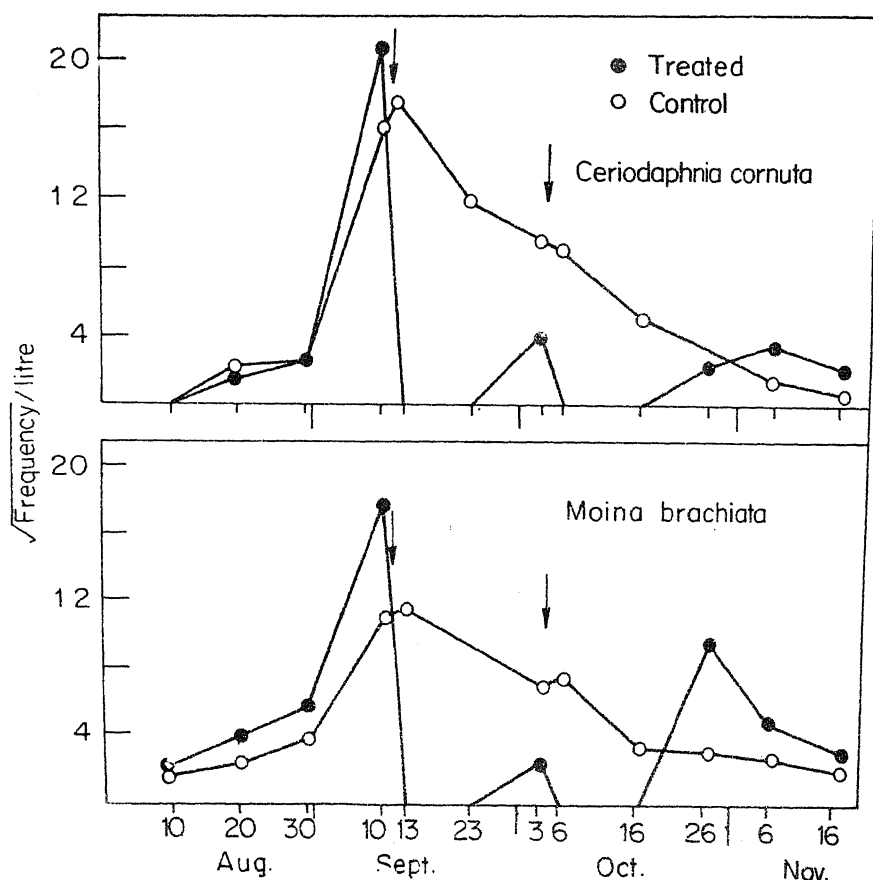


Figure 3. Effect of DDT treatment on the natural population of *Ceriodaphnia cornuta* and *Moina brachiata*. The arrows indicate the treatment day.

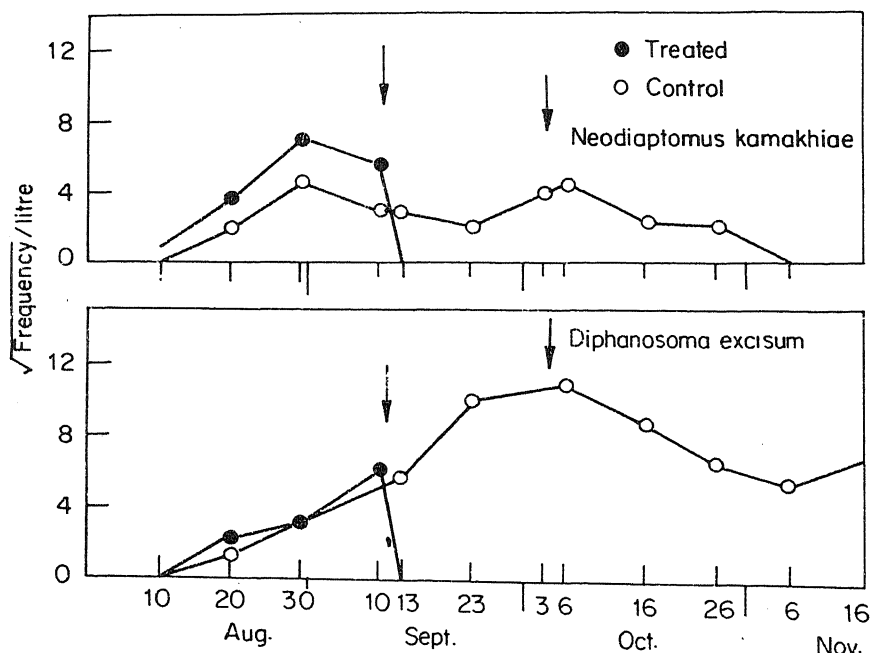


Figure 4. Effect of DDT treatment on the natural population of *Neodiaptomus kamakhiae* and *Diphanosoma excisum*. The arrows indicate the treatment day.

tank which received 1.0 lb/acre of Dursban treatment (Hurlbert *et al* 1970). One reason for such a failure on the part of certain plankters may be that the organism died owing to the insecticides treatment before they could produce any dormant eggs. It is also possible that the dormant eggs of *N. kamakhiae* and *D. excisum* are susceptible to DDT residues in the tank bed as in the present study (*vide infra*).

The population of cyclops, ostracods and larvae of *Anophelese* sp. was not affected by DDT treatment (figure 5). Didia *et al* (1975) and Ali and Mulla (1978) also found a number of insecticides extremely toxic to certain species of cladocerans but little or no toxicity to ostracods and copepods. The present results of the laboratory bioassay also indicate tolerance of *Cyclops* sp. to DDT (*vide supra*).

The variation of temperature and pH was almost similar in both the tanks (figure 6). However, a distinct maxima of dissolved O₂ content were observed in the sample of 23 September 1979 and 16 October 1979 respectively. These collections, which exhibited the maxima of dissolved O₂, were made after 12 days of each DDT treatment, and were probably due to increase in the population of phytoplankton and decrease in respiratory activity of microfauna. There are several reports which indicate the increase of phytoplankton population following the treatment of insecticides (Hurlbert 1975).

A bloom of aquatic weeds, *viz.*, *Wolfia* sp. and *Lamna* sp. was observed in the treated tank. These aquatic weeds proliferated during the period from 26 October to 6 November 1979. The water surface of the treated tank was thickly covered by these plants around 16 November when the present experiment was terminated. On the other hand these aquatic weeds were not observed in the control tank throughout

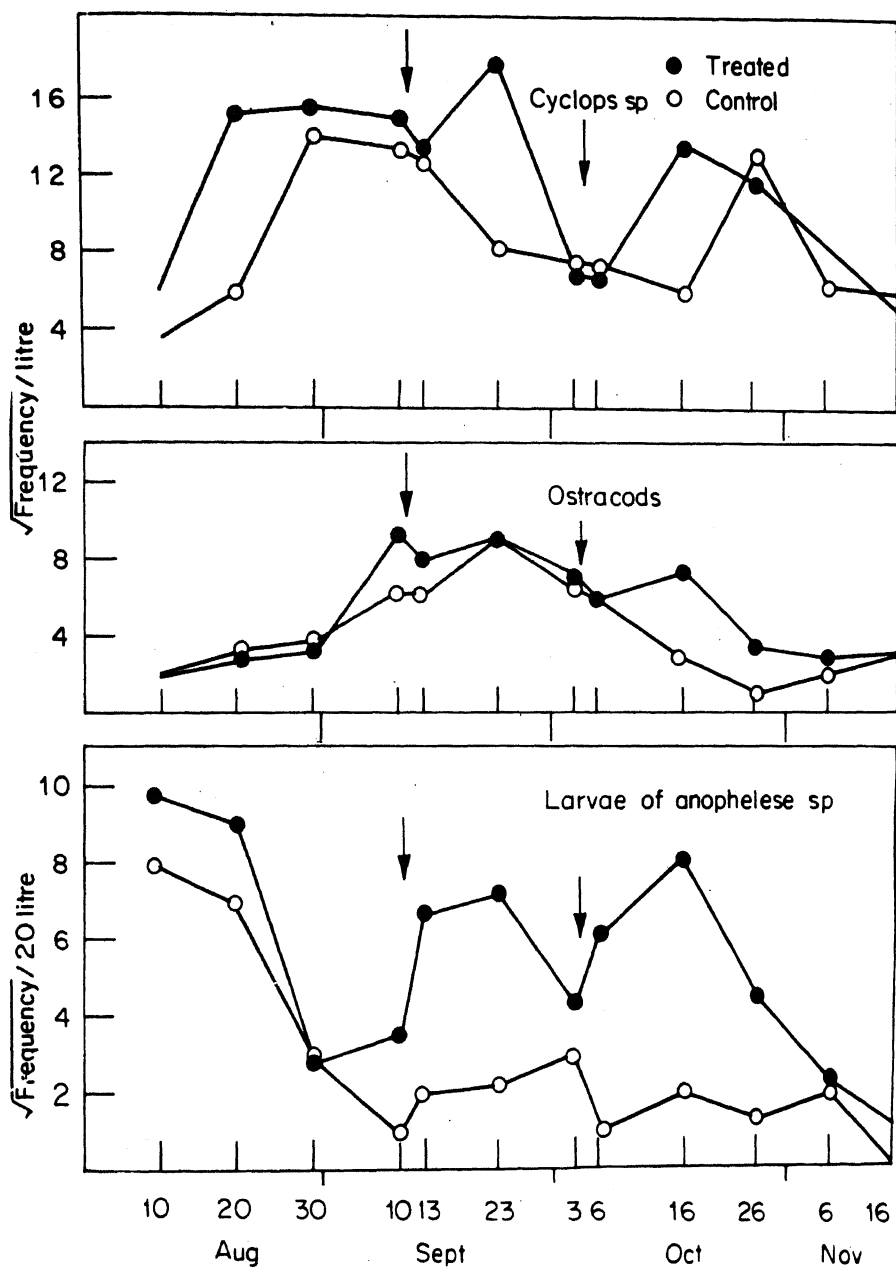


Figure 5. Effect of DDT treatment on the natural population of *Cyclops* sp., ostracods and larvae of *Anophelese* sp. The arrows indicate the treatment day.

the period of study. Similar blooms of aquatic macrophytes, viz., *Chara* and *Nitella* have been reported by other workers in their water bodies following herbicide or insecticide treatments (Walsh *et al* 1971; Brook and Edwards 1973).

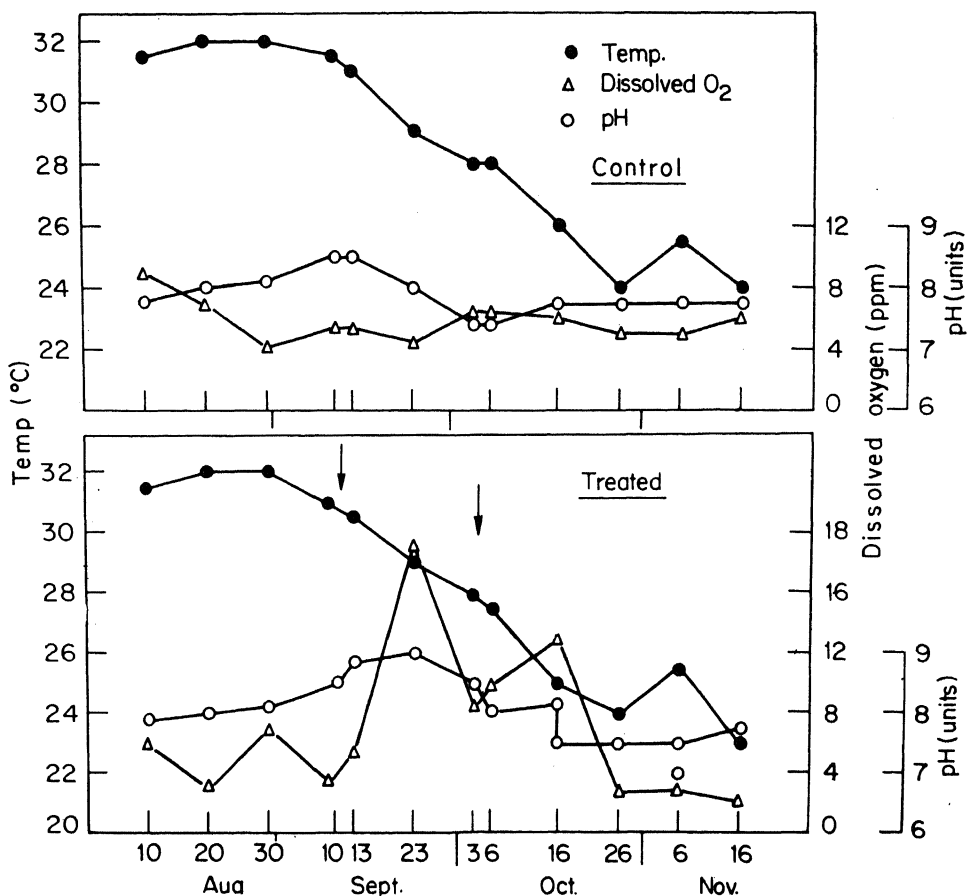


Figure 6. Physical and chemical characteristics of water in control and treated experimental tank.

The bottom soil of the treated tank revealed the presence of 36 ppb of DDD, 26 ppb of DDE and 3 ppb of DDT, whereas the soil of the untreated tank showed the presence of only traces of DDE, *i.e.*, 2 ppb (figure 7). Absorbed DDT in the soil from an aqueous dispersion has been reported as high as 80% within a period of 24 hr (Bowman and Schmidt 1961). It is interesting to note that although DDT was applied to the tank, it is the least abundant isomer found in the bottom mud. This indicates that DDT is probably degraded in water or in the bottom mud. The biodegradation of DDT to DDD and DDE by micro-organism present in water or soil has earlier been reported (Keil and Priester 1969; Johnson *et al* 1971).

4. Conclusion

The present paper provides basic information on DDT toxicity to some members of freshwater micrometazoans. *Neodiaptomus kamakhiae*, the most susceptible form

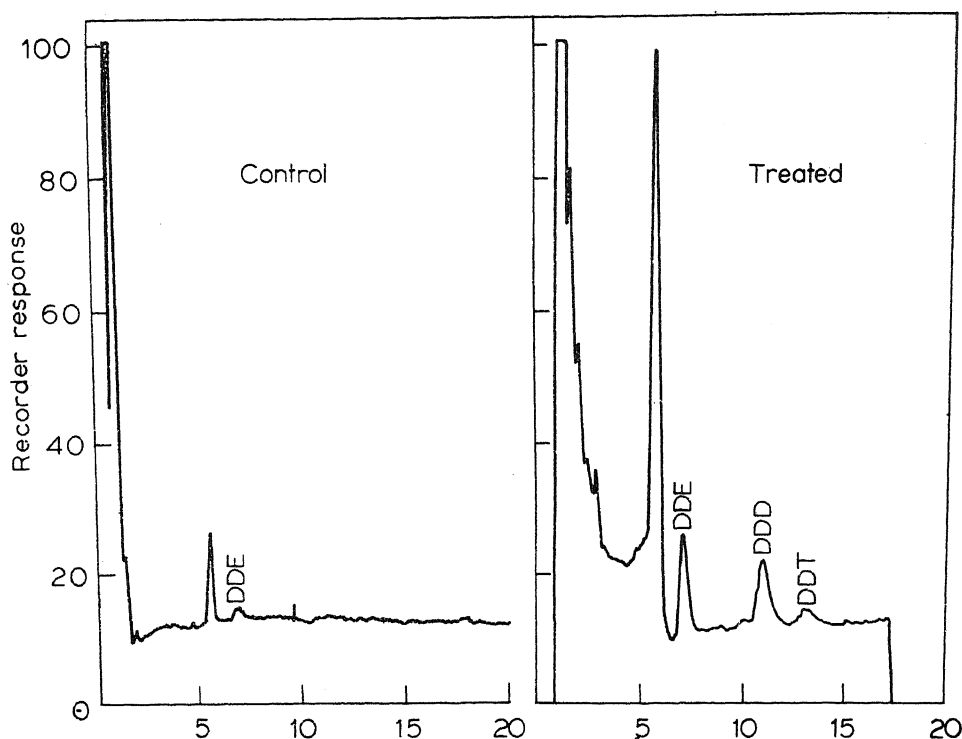


Figure 7. Gas chromatograms of the soil of the control and the treated experimental tank.

amongst the organisms tested during the present investigation could possibly be used to detect insecticide pollution in freshwater bodies. Apparently, the presence or absence of a particular type of zooplankton or the prolific growth of weeds now observed could indicate the nature and degree of pollution in the water.

Acknowledgements

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Effects of body size on the rate and pattern of ammonia excretion in an air-breathing fish

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Abstract. The rate and pattern of ammonia excretion of *Channa gachua* is described in relation to its body size. The food intake and rate of ammonia excretion was found to depend on the body size of the fish. The pattern of ammonia production is directly dependant on the food intake.

Keywords. *Channa gachua*; ammonia excretion; *Tubifex tubifex*.

1. Introduction

Ammonia is known to be the principal nitrogenous waste product excreted by freshwater fishes (Colt and Tohobanoglous 1976). Although freshwaters with alkaline pH are known to be highly productive and suitable for fish culture (Jhingran 1982), at 25 to 30°C, ammonia excreted by fish may accumulate to concentrations that may affect the food intake and growth of fish (Alderson 1979; Tomasso *et al* 1980; Jobling 1981). Several workers have studied the effects of temperature and meal size on the rate of ammonia excretion in fishes (Guerin-Ancey 1976a, b; Savitz *et al* 1977). Since feeding in fish is influenced by several endogenous factors including body size (Pandian 1975; Staples and Nomura 1976), the rate of ammonia excretion by fish may also be influenced by body size of fish (Jobling 1981). This paper describes the influence of food intake and body size on the rate and pattern of ammonia excretion in the freshwater air-breathing fish *Channa gachua*.

2. Material and method

Channa gachua (Ham.) were collected from a local freshwater tank and acclimated to the laboratory conditions for a week, during which they were fed on the oligochaete worms. Experimental fish were selected from this stock and based on their body size, were segregated into six series each with 5 individuals. The average body weights of fish in the six series were 0.432, 1.019, 2.878, 5.144, 9.880 and 10.975 g respectively. Each fish of a series was maintained individually in a glass aquarium (surface area: 729.95 cm²) containing 5 litre of ammonia free water and starved for 24 hr prior to the experiment. The fish in each series were subsequently fed on a known quantity of surplus food of *Tubifex tubifex* for 1 hr. At the end of the hour, the unfed food was recovered, weighed and the exact food intake of each fish was calculated and represented as mg food intake/fish/hr. The fish was then transferred to a fresh aquarium containing 5 litres of ammonia free water. During the subsequent 8 hr period, at intervals of every 2 hr, aliquot water samples were collected from

each aquarium and the ammonia content was determined following the method of Varley (1978). The experiment was repeated for each fish on three successive days and the data analysed statistically using a computer (model: Liverpool SPSS version 5 macro).

3. Results and discussion

3.1 Food intake

The average daily food intake of *Channa gachua* in relation to its body size is presented in table 1. Fish weighing 0.432 g consumed 154.8 mg/hr and this value increased to a maximum of 385.3 mg/hr as the body weight of the fish increased to 9.880 g. Beyond this body size, the food intake of fish decreased. When the food intake was expressed as g/g live fish/hr (= feeding rate), it was seen that fish weighing 0.432 g exhibited the maximum feeding rate of 0.341 g, while those weighing 9.880 g could feed only 0.039 g. Figures 1, 2 illustrate the inverse linear relationship

Table 1. Food intake and feeding rate of *Channa gachua* in relation to body size

Body size of fish (g)	Food intake (mg/fish/hr)	Feeding rate (g/g fish/hr)
0.432±0.065	154.8±0.015	0.341±0.012
1.019±0.110	198.7±0.024	0.195±0.043
2.878±0.397	296.4±0.052	0.103±0.011
5.144±1.033	272.6±0.012	0.053±0.013
9.880±0.669	385.3±0.132	0.039±0.012
10.975±0.877	263.4±0.122	0.024±0.016

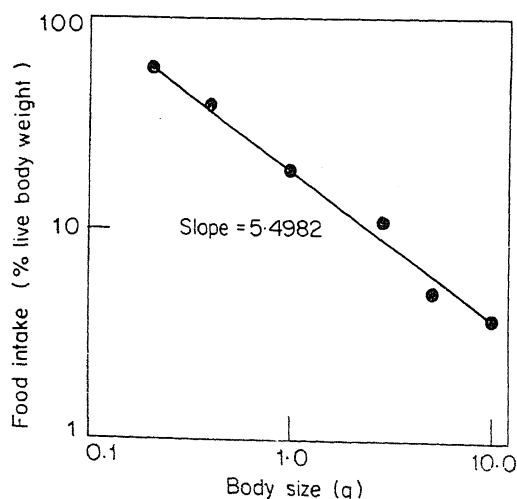


Figure 1. Relationship between the body size (X) and daily food intake (Y) in *Channa gachua*. Regression equation $Y = 58.65 - 5.4982X$

between food intake (expressed as percentage live body weight) and body size of *C. gachua*. It is seen that, for every 100 mg increase in the body weight of the fish, the feeding rate decreased by nearly 1%. Similar size correlated inverse feeding rates have also been observed in other channid fishes (Gerald 1976; Pandian 1967a).

Figure 2 gives the linear relationship between body weight of fish and ammonia excretion. With increase in body size of fish, the ammonia excretion was also found to increase. The rate of ammonia excretion in *C. gachua* as a function of body size is represented in table 2. From the table, it is apparent that smaller fish excrete more ammonia per g weight of the tissue than larger fish. Similar observations have also been made in the plaice *Pleuronectes platessa* (Jobling 1981). He reported that the relationship between rate of nitrogenous excretion and fish size takes the form of $M = aw^b$ (where m =rate of nitrogenous excretion, w =fish size, b is the weight exponent and a is the y -axis intercept), clearly indicating that nitrogenous

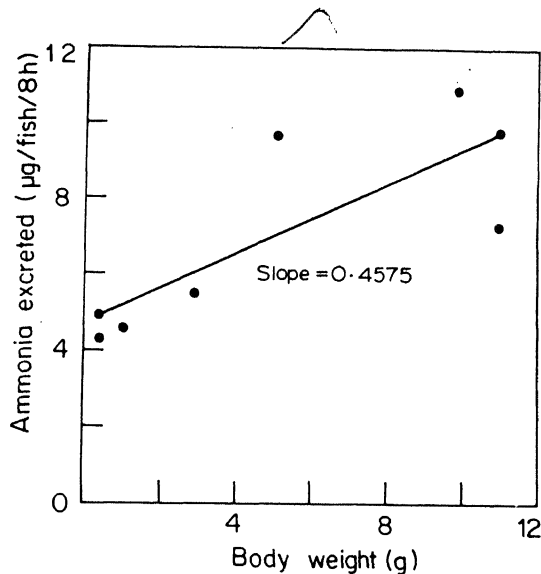


Figure 2. Relationship between the body weight (X) and rate of ammonia excretion (Y) in *Channa gachua*. Regression equation $Y = 4.7293 + 0.4575X$.

Table 2. Rate of ammonia excretion by *Channa gachua* in relation to body size

Body size of fish (g)	Ammonia excretion ($\mu\text{g/g fish/8hr}$)
0.432 ± 0.065	9.84 ± 1.324
1.019 ± 0.110	4.55 ± 0.645
2.878 ± 0.397	1.98 ± 0.128
5.144 ± 1.033	1.89 ± 0.204
9.880 ± 0.669	1.10 ± 0.076
10.975 ± 0.877	0.66 ± 0.163

Table 3. Pattern of ammonia excretion in *Channa gachua* during the 8 hr period.

Body size of fish (g)	Ammonia excreted ($\mu\text{g/g}$ fish)			
	2 hr	4 hr	6 hr	8 hr
0.432 $\pm$ 0.065	5.21 $\pm$ 0.295	2.33 $\pm$ 0.784	0.89 $\pm$ 0.441	1.41 $\pm$ 0.196
1.019 $\pm$ 0.110	1.60 $\pm$ 0.436	1.09 $\pm$ 0.413	0.85 $\pm$ 0.042	1.01 $\pm$ 0.012
2.878 $\pm$ 0.397	0.71 $\pm$ 0.176	0.54 $\pm$ 0.101	0.19 $\pm$ 0.052	0.55 $\pm$ 0.086
5.144 $\pm$ 1.033	0.59 $\pm$ 0.097	0.40 $\pm$ 0.155	0.43 $\pm$ 0.168	0.47 $\pm$ 0.055
9.880 $\pm$ 0.669	0.36 $\pm$ 0.069	0.18 $\pm$ 0.049	0.31 $\pm$ 0.034	0.25 $\pm$ 0.026
10.975 $\pm$ 0.877	0.22 $\pm$ 0.083	0.22 $\pm$ 0.096	0.03 $\pm$ 0.000	0.20 $\pm$ 0.040

metabolism and excretion could reflect general trends in the overall metabolic rate of the fish. The present results on *C. gachua* expressed in a similar way indicate that the weight exponent is 0.46. Winberg (1960) considered that a weight exponent as high as 0.81 describes the metabolic rate-size relationship of fishes. Although such values relating nitrogenous excretion to body size of fishes have been reported (von Infant 1974; Guerin-Ancey 1976a), some workers have found either higher (Savitz 1969; Iwata 1970) or lower (Gerking 1955a; Jobling 1981) values.

The pattern of ammonia excretion of *C. gachua* in relation to its body weight is represented in table 3. It is interesting to note that irrespective of the body weight of the fish, the rate of nitrogenous excretion increased markedly shortly after food intake and then declined subsequently. In the blue gill sunfish *Lepomis macrochirus*, Savitz (1971) has indicated a direct relationship between rate of nitrogen excretion and activity oxygen consumption. Since it is known that in fish the metabolic activity increases following feeding (Brett and Zala 1975) the high rate of ammonia production observed in *C. gachua* soon after feeding may be due to the increased metabolic activity, higher oxygen uptake and/or increase in the level of SDA consequent to digestive processes (see Brody 1968).

Thus it may be concluded that in *C. gachua* the rate of ammonia excretion is strongly influenced by body size and that the pattern of ammonia production is dependant on ingestion of food.

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Distribution pattern of succinic dehydrogenase in the myocardia of some vertebrates

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Abstract. A comparative study on the myocardial SDH levels in certain representative vertebrates revealed interesting variations. The *Cybbium* myocardium exhibited relatively lesser SDH levels, whereas *Rana*, despite its diving habits, had a higher level with significant variations between the two ventricular halves. *Calotes*, an active terrestrial form, showed the highest enzyme level among the reptiles. The SDH activity of the avian myocardia are quite high, with *Columba* having a greater level than *Gallus*. The myocardia of the actively flying forms (*Columba* and *Pteropus*) exhibited higher enzyme levels in the right ventricle than the left ventricle.

Keywords. Succinic dehydrogenase; vertebrate myocardia; myocardial metabolism; oxygen requirements; chamberwise variations; activity levels; flying habit; diurnal dormancy.

1. Introduction

The vertebrate heart is a multi-chambered organ, exhibiting specific regional variations in its contractile performance facilitated by various factors (Rubel 1968). The morphology of the heart of several vertebrate groups has been observed to be correlated to the specific functional adaptation of each animal (Johansen and Hansen 1968; Randall 1968; White 1968, 1976). Considerable attention has been focussed on the biochemical basis of cardiac function in mammals (Balasubramanian *et al* 1973; Dhalla *et al* 1972, 1974; Tomlinson and Dhalla 1973). However, relatively little information is available on the biochemical adaptation of the vertebrate myocardia to diverse physiological conditions. Specific chamberwise variations with regard to mitochondrial activity and other parameters in the myocardia of several mammals have been reported (Laguens 1971; Alexander 1975; Sordahl 1976). The present study is an attempt to elaborate some aspects of the enzymic variations in the different chambers of the myocardia of some representative vertebrates having varied ecological conditions and activity levels, with special reference to one of the key enzymes of cardiac metabolism, namely succinic dehydrogenase.

2. Material and methods

The myocardia of nine vertebrates belonging to various classes and activity levels have been chosen for the present study. A list of animals with their habitat is given in table 1. Healthy adult animals were collected from their natural habitats and myocardial samples of the various chambers of these vertebrates were carefully

excised. A tissue homogenate was prepared in double distilled water at 4°C. SDH activity was estimated by the colorimetric method of Kun and Abood (1949). The reaction was carried out in phosphate buffer at pH 7.4 using 0.4 M sodium succinate as substrate and 0.1% solution of triphenyl tetrazolium chloride as the electron acceptor.

3. Results

The data obtained for the SDH activity of the various chambers of the myocardia of the vertebrates investigated are given in table 2.

Relatively a lower level of the SDH activity is discernible in the myocardia of *Cybiurn* than that of *Rana*, but it is greater than that of the myocardia of the chelonians. The SDH activity of the left atrium and left ventricle of *Rana* is significantly higher than that of the right atrium and right side of the ventricle respectively. Among the reptiles, higher enzyme level is observed in the myocardia of *Calotes* with preponderance of the SDH activity in the right ventricle than the left ventricle. The difference in the enzyme activity of the two ventricular halves of *Lissemys* is negligible. The avian myocardia also exhibit higher SDH activity, and among the two birds investigated, a higher enzyme level was observed in the myocardia of the actively flying form *Columba*. With regard to mammals, the atria of *Pteropus* possess a relatively higher SDH activity than that of *Capra*. The right half of the myocardia of *Columba* and *Pteropus* show a greater SDH activity than the left.

4. Discussion

Succinic dehydrogenase being an oxidative enzyme has considerable relevance in the energy metabolism of the cardiac muscle. Irrespective of the source of the fuel, oxidative metabolism takes place in the mitochondrion through the citric acid cycle. In fact data on SDH would facilitate a better insight into the metabolism of the cardiac muscle of the various animals investigated.

Table 1. Animals used and their habitats

Class	Animal	Full name	Habitat
Pisces	Seer fish	<i>Cybiurn guttatum</i>	Marine (actively swimming)
Amphibia	Frog	<i>Rana tigrina</i>	Amphibious
Reptilia	Turtle	<i>Lissemys punctata</i>	Fresh water (diving)
	Tortoise	<i>Geomyda trijuga</i>	Terrestrial-moist surroundings
	Garden lizard	<i>Calotes versicolor</i>	Terrestrial
Aves	Pigeon	<i>Columba livia</i>	Terrestrial (actively flying)
	Domestic fowl	<i>Gallus domesticus</i>	Terrestrial (very poor flier)
Mammalia	Goat	<i>Capra</i> sp.	Terrestrial
	Bat	<i>Pteropus giganteus</i>	Terrestrial (flying)

The SDH activity of the myocardium of *Cybbium* is lower than that of some of the warm blooded vertebrates. The oxygen requirements for the fish is generally a fraction of those for higher vertebrates (Nicol 1960). The lower SDH activity of the myocardium of *Cybbium* may possibly be indicative of this. Between the two myocardial chambers of *Cybbium*, the atrium has a higher activity than the ventricle. Randall (1968) has attributed a more significant role to the atria, based on the similarity of the atrial and ventricular volume of fish, in the ventricular filling and the higher SDH level of *Cybbium* may be attributed to the specific haemodynamic demands of the chamber.

Interestingly enough the heart of *Rana* has a relatively higher SDH activity. In frogs, the skin, in addition to the lungs, serve as a respiratory surface. The total gaseous uptake through the lungs and skin might provide an ample supply of oxygen to the myocardial tissue. This oxygen uptake may be sufficient for the higher oxidative metabolism of the cardiac muscles of *Rana*, as indicated by the SDH activity. Significant variations have been observed between the two atria and between the two halves of the ventricle. Such a specific variation, though the ventricle of *Rana* is single chambered, is interesting. Johansen and Hansen (1968) have reported a trabecular network in the ventricle of amphibians, which runs in an

Table 2. Distribution pattern of SDH in the myocardia of various vertebrates (μ gm of formazan/min/gm of wet tissue)

	Atrium		Ventricle	
	Right	Left	Right	Left
<i>Cybbium guttatum</i>	28.36 (0.89)		16.79 (0.66)	
<i>Rana tigrina</i>	32.99 (1.81)	49.74 (3.05)	40.67 (3.23)	56.05 (3.94)
<i>Lissemys punctata</i>	10.51 (0.56)	12.36 (0.21)	16.18 (0.86)	17.27 (0.79)
<i>Geomyda trijuga</i>	18.81 (0.95)	13.12 (0.94)	11.27 (0.64)	15.86 (1.04)
<i>Calotes versicolor</i>	60.28 (2.03)	67.23 (2.23)	97.23 (2.09)	89.11 (4.08)
<i>Columba livia</i>	78.27 (2.22)	65.88 (5.63)	109.18 (8.61)	90.79 (7.85)
<i>Gallus domesticus</i>	40.27 (2.75)	49.64 (4.43)	59.46 (4.33)	66.87 (5.25)
<i>Capra</i> sp.	15.78 (1.25)	20.91 (0.69)	27.28 (2.57)	36.04 (3.28)
<i>Pteropus giganteus</i> *	59.16 (3.48)	46.55 (2.57)	27.98 (1.61)	17.29 (0.89)

(Values are the mean of ten assays, with standard error in brackets)

Analysis of variance was carried out and the F ratio was calculated to test the significance of variations. Significant variations were observed between animals ($p < 0.01$) and between chambers ($p < 0.01$)

* samples were taken during day time only due to practical difficulties.

antero-posterior direction, maintaining a right and left compartmentalisation. The variation in the SDH activity in the two halves of the ventricle may possibly be due to this phenomenon.

Among the reptiles investigated, *Calotes* myocardia exhibit relatively higher levels of the SDH activity indicating a higher oxidative metabolism than those of *Geomyda* and *Lissemys*. *Calotes* is a terrestrial reptile with access to plenty of atmospheric oxygen and has relatively more active habits, the higher energy demands can be efficiently met only by an increased level of oxidative metabolism. Significant chamberwise variations are also noticed. The left atrium has a higher enzyme level than the right, and the right half of the ventricle has a higher enzyme activity than the left half.

The myocardia of the two chelonians studied exhibited only relatively low SDH activity. *Geomyda* is an extremely sluggish form whereas *Lissemys* is a relatively active diving form. However, during submergence, *Lissemys* is exposed to limited supply of oxygen. Our studies on the fuel reserves (unpublished data) have shown a very high level of glycogen in the myocardia of *Lissemys*. Hence quite probably during diving, its myocardia may depend more on anaerobic glycolysis. In the case of *Geomyda*, on account of its extremely sluggish activity pattern, the total circulatory demand may be reduced. Presumably this might have induced a lower energy demand in the myocardia of *Geomyda* which is reflected by a lower level of SDH activity.

The birds by virtue of their higher metabolic rate have relatively higher SDH activity, with *Columba* myocardia having a greater level than that of *Gallus*. Talesera and Narang (1979) have also reported a higher SDH activity in the cardiac muscles of *Columba* (7.68) as compared to that of *Gallus* (5.08). However, these values are for the whole heart, rather than for the different myocardial chambers. The present study reveals interesting chamberwise variations. The right atrium and right ventricle of *Columba* exhibit higher SDH levels than those of the left atrium and left ventricle, indicating a higher metabolic activity in the right half than the left. It may be noted here that the right half, viz., the right atrium and the right ventricle, is concerned with the pulmonary circulation. The active flying habits of *Columba* necessitates a greater demand of oxygen which can be met to a certain extent by an increased blood supply to the lungs. Hence the higher metabolic activity of the right myocardial half may possibly be due to this specific vascular demand.

Among the mammals investigated, lower values have been obtained for the myocardia of *Capra*. Laguens (1971) reported a higher numerical and volumetric density of mitochondria in the left ventricle of the rat than the right ventricle. In the present study also, SDH, a key mitochondrial enzyme did reveal a significant variation between the two ventricles of *Capra* with left ventricle showing a higher value than the right.

A comparative study on the myocardial SDH levels of some birds and mammals have revealed a higher enzymic level in the heart of bat *Pipistrellus* sp. (11.60) as compared to that of pigeon, chicken and rat (Talesera and Narang 1979). However, in the present study the myocardia of *Pteropus* exhibited lower SDH level than *Columba*. According to Kulzer (1967) a reduction in heart rate occurs in the daily torpor of bats. *Pteropus* also exhibits diurnal dormancy and probably the lower SDH level may be reflection of the metabolic adaptation of its myocardia to diurnal dormancy. The chamberwise distribution pattern of SDH in the myocardia of

Pteropus shows a similarity to that of *Columba*. In both cases a greater level of SDH activity is observed in the right myocardial half than the left, which may be an adaptation in correlation to the greater cardiovascular demands due to their flying habit.

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Food preference studies of the larger bandicoot rat, *Bandicota indica* (Bechstein)

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Abstract. The food preferences of the South-Asian commensal rat, *Bandicota indica* were studied to evolve a suitable bait for control. Amongst cereals clear preference towards rice in its whole/divided form was evident. Amongst additives 7-10% groundnut oil enhanced bait intake significantly while 2% salt and sugar did not influence the rat's diet choice. The results indicate whole/broken rice mixed with 7-10% groundnut oil as the most attractive bait for poisoning *B. indica*.

Keywords. Food preferences; cereals; pulses; oils; calorie intake; best bait; *Bandicota indica*.

1. Introduction

The larger bandicoot rat, *Bandicota indica* (Bechstein) is widely distributed in Burma, Vietnam, Thailand, Indonesia, Formosa, Hongkong and almost the whole of India. Unlike the field and forest inhabiting lesser bandicoot rat, *Bandicota bengalensis*, the larger bandicoot is commensal, living close to buildings, gardens, stables and out houses. To a small extent it is reported to inhabit the warehouses of Calcutta (Spillet 1968). Though no match to the number and extent of infestation of house rat (*Rattus rattus*), larger bandicoots do become as commensal as the former, particularly in South India. They are omnivorous depending on domestic refuse, grains and vegetables and occasionally attack poultry. Much damage is inflicted by the species to ground, flooring and crop by their burrowing habits. Its ecology and biology are known to a very small extent (Spillet 1968; Arjunwadkar and Gadgil 1974). The effective control of *B. indica*, as in the case of other rodent pests, necessitates formulation of highly attractive and specific baits as poison carriers. In this paper we describe its food preferences based on laboratory experiments.

2. Material and methods

2.1 Subjects

Bandicota indica were trap collected at Hesaraghatta and brought to laboratory. They were confined singly in metal cages and fed on *ad lib* quantities of standard rat and mouse feed and water prior to food preference tests.

2.2 Feeding tests

Except for four cereals tested individually as single choice tests, the rest of the experiments were in the nature of multiple choice tests with a maximum of five and

minimum of three candidate baits. In each test 50 g of candidate foods were offered each day and consumptions recorded 24 hrs later. Grain spilled outside the cages were collected and deducted from this to arrive at exact amounts eaten. Each test ran for seven continuous days. The scheme of tests was as follows: Test I to IV—single choice of cereals; Test V—multiple choice of cereals; Test VI—multiple choice of pulses; Test VII—texture preference of best liked cereal; Test VIII to X—different concentrations of groundnut, coconut and gingilly oil mixed with broken rice; Text XI—oil preference; Text XII—addition of 2% sugar and salt on bait intake; Test XIII—best bait composition arrived at *vs* plain rice *vs* plain wheat.

2.3 Statistics

Daily consumptions were converted to g consumed/100 g body weight and their means with standard error computed. In multiple choice tests percent consumption of each candidate diet were compared. Preferential order of bait acceptancy (ranks) was established after conducting Kruskal-Wallis one way analysis of Variance (Seigel 1956).

The calorie equivalents of foods consumed were calculated and compared during the various choice tests. Student 't' tests were performed to compare the highest and lowest calorie ingested during tests as well as to compare average daily intake of food during single and multiple choice tests of cereals.

3. Results

3.1 Cereal and pulse preference

The mean daily intake of rice, ragi, jowar and wheat was not significantly different from each other during single choice test (table 1). The larger bandicoot rat ate 3-4 g of the given cereal/100 g body weight/day. However, the animal exhibited preferential order of cereal intake during multiple choice test only. This order was rice > jowar > wheat > maize > ragi (table 1). Similarly the order of pulse intake was groundnut > greengram > cowpea > field bean (table 1).

3.2 Texture preference

Whole grain and broken form were preferred to rice in powder form (table 1).

3.3 Oil preference

The order of consumption of different concentrations of groundnut oil was 10% > 7% > 1% > 3% > 5%, that of coconut oil was 10% > 7% > 5% > 3% > 1% and gingilly oil was liked in the order of 10% > 7% > 1% > 3% > 5%. Amongst the three oils the preference was groundnut > gingilly > coconut (table 1).

3.4 Additives

Addition of salt and sugar at 2% did not affect the rate of bait consumption in larger bandicoots (table 1).

3.5 Best bait composition arrived at *vs* rice *vs* ragi

The bait composition arrived at after the various preference tests namely cracked

rice + 10% groundnut oil was definitely consumed more than plain cereals *viz.* rice and wheat (table 1).

3.6 Calorie intake

The mean calorie intake during the different food tests did not differ significantly and varied from 13.34 ± 0.24 to 26.65 ± 5.23 calories (table 2).

3.7 Single and multiple choice test

Although the rate of consumption did not differ in the two types of choice test ($p < 0.2$), the preferential order was evident only in the multiple choice test (table 1).

4. Discussion

4.1 Bait preference

In all the 13 tests conducted involving various foods and additives, the rats sampled considerable amount of all foods in addition to the large quantity of preferred food. Amongst the cereals rice was the most liked as in the case of lesser bandicoot rat, *Bandicota bengalensis* (Harrison and Woodville 1950; Sridhara and Krishnamoorthy 1968). Amongst pulses groundnut was preferred to the others. Rice either as whole cereal or in cracked form was preferred to flour form, an observation contrary to the preferential consumption of finely divided/powder form of cereals by majority of the rodents (Khan 1974; Jain *et al* 1974; Prakash *et al* 1975). Only *Rattus cutchicus* is known to exhibit a similar taste for whole/cracked cereal (Jain *et al* 1975). Even

Table 2. Comparison of calorie intake during different choice tests.

Choice tests for	Total calorie intake/100 g $\pm$ S.E.
1. Cereals : a) Single choice — rice	13.34 $\pm$ 0.24
ragi	13.94 $\pm$ 0.49
jowar	13.37 $\pm$ 0.28
wheat	13.7 $\pm$ 0.28
b) Multiple choice	14.65 $\pm$ 2.72
2. Pulses	19.57 $\pm$ 3.06
3. Different textures of rice	15.87 $\pm$ 3.59
4. Different concentrations of groundnut oil in cracked rice	24.42 $\pm$ 4.95
5. Different concentrations of coconut oil in cracked rice	17.89 $\pm$ 3.58
6. Different concentrations of gingilly oil in cracked rice	20.34 $\pm$ 3.96
7. Cracked rice with 10% groundnut oil vs same with 10% coconut oil vs same with 10% gingilly oil	19.71 $\pm$ 4.31
8. Cracked rice with 10% groundnut oil vs same plus 2% sugar vs same with 2% salt	25.65 $\pm$ 6.23
9. Cracked rice with 10% groundnut oil (best bait arrived at) vs rice vs wheat	19.09 $\pm$ 2.82

Table 1. Bait preference of *B. indica*.

Bait choice	A.D.I. $\pm$ S.E. (g/100g)	Percent eaten	Preferential rank
<i>Cereals—single choice</i>			
I. Rice	3.88 $\pm$ 0.07	—	—
II. Ragi	4.25 $\pm$ 0.15	—	—
III. Jowar	3.83 $\pm$ 0.08	—	—
IV. Wheat	3.96 $\pm$ 0.08	—	—
V. <i>Cereals—multiple choice</i>			
a) Rice	2.25 $\pm$ 0.34	52.8	1
b) Ragi	0.39 $\pm$ 0.09	9.2	4
c) Jowar	0.76 $\pm$ 0.22	17.8	2
d) Maize	0.37 $\pm$ 0.06	8.7	3
e) Wheat	0.49 $\pm$ 0.08	11.5	3
VI. <i>Pulses</i>			
a) Green Gram	1.66 $\pm$ 0.49	36.4	2
b) Cowpea	0.78 $\pm$ 0.05	17.1	3
c) Field bean	0.08 $\pm$ 0.03	1.75	4
d) Groundnut	2.04 $\pm$ 0.21	44.7	1
VII. <i>Particle size of most preferred cereal (rice)</i>			
a) Whole	1.62 $\pm$ 0.26	35.2	1
b) Cracked	2.25 $\pm$ 0.39	48.9	1
c) Flour	0.73 $\pm$ 0.18	15.9	2
VIII. <i>Oil preference: different concentrations of groundnut oil with cracked rice</i>			
a) 1%	1.32 $\pm$ 0.34	21.6	5
b) 3%	0.88 $\pm$ 0.23	14.3	4
c) 5%	0.65 $\pm$ 0.17	10.5	3
d) 7%	1.46 $\pm$ 0.18	23.7	2
e) 10%	1.85 $\pm$ 0.35	29.9	1
IX. <i>Oil preference: different concentrations of coconut oil mixed with cracked rice</i>			
a) 1%	0.18 $\pm$ 0.15	4.1	5
b) 3%	0.29 $\pm$ 0.13	6.7	4
c) 5%	0.46 $\pm$ 0.15	10.6	3
d) 7%	1.56 $\pm$ 0.18	36.0	2
e) 10%	1.85 $\pm$ 0.29	42.6	1
X. <i>Oil preference: different concentrations of gingelly oil mixed with cracked rice</i>			
a) 1%	0.90 $\pm$ 0.3	17.7	3
b) 3%	0.61 $\pm$ 0.13	12.0	4
c) 5%	0.65 $\pm$ 0.09	12.7	4
d) 7%	1.18 $\pm$ 0.18	23.2	2
e) 10%	1.75 $\pm$ 0.31	34.4	1
XI. <i>Oil preference: 10% groundnut vs 10% coconut vs 10% gingelly oil mixed with cracked rice</i>			
a) Groundnut	2.14 $\pm$ 0.35	47.2	1
b) Coconut	0.92 $\pm$ 0.16	20.3	3
c) Gingilly	1.47 $\pm$ 0.46	32.5	2
XII. <i>Effect of sugar and salt on bait intake</i>			
a) Plain bait	1.28 $\pm$ 0.31	27.7	—
b) Bait + 2% sugar	1.56 $\pm$ 0.46	33.7	—
c) Bait + 2% salt	1.79 $\pm$ 0.29	38.6	—
XIII. <i>Best bait composition vs rice vs wheat</i>			
a) Best bait	2.57 $\pm$ 0.25	52.9	1
b) Rice	1.21 $\pm$ 0.29	24.9	2
c) Wheat	1.08 $\pm$ 0.21	22.2	2

Values are mean of 20 observations $\pm$ S.E.

larger-sized cereals like jowar, maize and wheat were consumed more by *B. indica* than small-sized ragi.

4.2 Single and multiple choice test

The average daily intake of food was not different in single and multiple choice test although Bindra and Sagar (1970) reported higher intakes during multiple choice test in *Rattus meltda*, *Tatera indica* and *Bandicota bengalensis* and Prakash *et al* (1975) observed lower rates of food intake under similar conditions for *Gerbillus gleadowii*. However, no significant variation was detected in food intake between single and multiple choice tests for *Rattus rattus* (Krishnamurthy *et al* 1967; Deoras 1968), *Mus musculus* (Krishnamurthy *et al* 1967) and *Rattus meltda* (Jain *et al* 1974).

4.3 Additives

Of the three oils tested addition of 10% groundnut oil to the bait enhanced consumption more than the other oils. Similar enhancement of bait intake due to addition of vegetable oils has been reported for black rats by Khan (1974) and for desert gerbils by Prakash *et al* (1969).

Although sugar/saccharin addition elevated the levels of food ingestion in house and Norway rats (Khan 1974; Barnett and Spencer 1953), Prakash *et al* (1975) did not observe any such trend when 2% salt/sugar was added to the diet of *Gerbillus gleadowii*. Similarly *B. indica* did not show any significant liking towards food containing salt/sugar.

4.4 Calorie intake

Like other mammals *B. indica* also regulated its calorie intake depending on the energy value of food. The mean calorie intake did not vary significantly during the different choice tests.

5. Conclusions

From the results and discussion it is evident that broken rice mixed with 10% groundnut oil can be effectively used as bait for controlling the larger bandicoot rat, *Bandicota indica*.

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A sensitive period for the acquisition of food preferences in the Indian field mouse *Mus booduga* (Gray)

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Abstract. The effect of exposure to particular cereals during infancy on adult food selection, and the sensitive period of acquisition of such food preferences, were studied in the Indian field mouse, *Mus booduga* Gray. Preliminary tests indicated an influence of early exposed diet on adult food selection. Further tests suggested that there is a sensitive period (4–6 days after birth) during which long lasting diet preferences are established in this wild mouse.

Keywords. Early experience, food selection, sensitive period, *Mus booduga* Gray, Indian field mouse

1. Introduction

Foods ingested early in life have a profound influence on adult diet selection in the snapping turtle, *Chelydra serpentina* (Burghardt and Hess 1966), the diamond terrapin, *Malaclemys centrata* (Allen and Littleford 1955) and sheep (Arnold and Maller 1977). Garter snakes displayed enhanced responsivity to the odour of infant experienced diet, although such preferences were easily alterable (Fuchs and Burghardt 1971). The evidence for food imprinting in birds is overwhelming (Hess 1964; Burghardt 1969; Capretta 1969; Hogan 1977). It is difficult to demonstrate the primacy effect of such olfactory or food experience in rodents. Earlier attempts by Warren and Pfaffmann (1959), Krishnakumari (1973) and Bronson (1966) failed to show any effect of foods ingested during post weaning on adult food selection in rats and guinea pigs although recent work of Capretta and Rawls (1974), Bronstein and Crockett (1976), Cornwell (1976), Porter and Etscorn (1976) and Sridhara (1978) showed that food odour and flavours experienced very early in life *i.e.* during preweaning or during the first post natal week of life result in temporary preference towards such odours and flavours.

In the present study tests were carried out to determine the effect of exposure to cereals during preweaning, on diet selection during adulthood and the timing of any sensitive period that there may be for the development of food preferences in the Indian field mouse, *Mus booduga* Gray. The species is a widely distributed pest of agricultural crops causing considerable damage (Barnett and Prakash 1975). In formulating effective baits for its control, it is helpful to consider factors influencing its feeding behaviour, hence this study.

2. Materials and methods

The subjects were caught by digging burrows in cultivated fields. Pairs of adult mice

were maintained in laboratory cages ($30 \times 25 \times 30$ cm) made of sheets and mesh of galvanized iron, with two nest boxes ($9 \times 12 \times 9$ cm), one at each end containing hay and cottonwool for bedding. The animals were maintained on a 12:12 LD schedule, with the light period beginning at 0600 hours each day. The room temperature was $25 \pm 2^\circ\text{C}$. During pregnancy and upto the birth of the litter, females were fed *ad lib* rat and mouse feed (Hindustan Lever, India), a standard unattractive lab chow and water. The gestation and weaning periods are 21 and 28 days respectively.

The first experiment was designed to detect the influence of food ingested early in life on diet selection in adulthood. The naive litters of three different mothers were exposed to three different foods, namely rice, ragi and wheat until weaning. During the following fortnight, the three experimental batches received rat and mouse food. On the sixteenth day, the rice-fed group was exposed to a choice of rice versus jowar, the ragi-fed batch were given ragi and jowar, and the wheat-fed batch were given wheat and jowar. After 24 hr, the relative consumption of the two choice foods was measured to the nearest 0.1 g. Following the preference test, the three experimental groups were fed on commercial rat and mouse feed for 15 consecutive days. A second choice test, identical to the first, was conducted at the end of this period.

The aim of the second experiment was to detect if any, the sensitive period for food preference acquisition. Naive litters from at least two mothers were used for each batch. The experiment involved exposing litters of different age groups to a specific food for a fixed period of 3 days at each age. Accordingly a total of seven age groups, starting at ages 1 day, 4 days, 7 days, 10 days, 13 days, 16 days and 19 days (table 1) were exposed to a rice-ragi mixture (50:50 by weight) for 3 days and then maintained on commercial rat feed for the next 15 days. Apart from these one more batch of one-day old litters was exposed to rice-ragi mixture for a day. The above groups were then given a test involving choice between the rice-ragi mixture and jowar. The intake of the two foods was measured to the nearest 0.1 g 24 hr later. The choice tests were repeated a fortnight and a month later. The subjects were given commercial rat and mouse feed before and after food conditioning and in the interval between the choice tests; water was available *ad lib* throughout the study period.

Table 1. Summary of the design of experiment 2.

	Batch No.							
	1	2	3	4	5	6	7	8
No. of animals in batch	9	8	9	7	8	8	10	8
Age of litter at initial exposure to rice-ragi mix. (days after birth)	1	1	4	7	10	13	16	19
Choice test 1*	15 days after initial exposure							
Choice test 2*	32 days after initial exposure							
Choice test 3*	48 days after initial exposure							

* rice-ragi mixture versus jowar

The cereal consumptions in experiment 1 were compared statistically using student *t* tests. In the second experiment, the percentage by weight of the food eaten that consisted of the rice-ragi mixture were calculated and subjected to an overall analysis of variance (Snedecor and Cochran 1968). In addition, an ANOVA was also carried out for comparison of the eight age groups in each of the three choice tests.

3. Results

The mice of the three experimental groups of test one preferred rice, ragi and wheat, the cereals to which they had respectively been pre-exposed, to the novel jowar ($P < 0.001$, table 2). A similar preference was observed during the second choice test with the earlier exposed cereals being consumed much more than the more recently experienced jowar ($P < 0.001$, table 2).

Significant variation was observed in the intake of rice-ragi mixture and jowar between the eight age groups ($F = 41.12$, $df = 7$, $P < 0.01$; table 3), between the three tests ($F = 72.96$, $df = 2$, $P < 0.01$), and for the age/test interaction ($F = 10.83$, $df = 14$, $P < 0.001$). The influence of training age on adult diet choice was confirmed by a separate one way analysis of variance across the eight age categories for each of the choice tests (test 1: $F = 36.65$, $df = 7$, $P < 0.01$; test 2: $F = 10.58$, $df = 7$, $P < 0.01$; test 3: $F = 16.76$, $df = 7$, $P < 0.01$). Early experience influences later food selection only in the groups trained on days 1-3, 4-6 and 7-9 in the first test (CD value = 14.32 at $P < 0.01$), in the mice trained on days 1-3 and 4-6 in the second test (CD value = 13.47 at $P > 0.05$) and in the 10-12 days (CD value = 20.07 at $P < 0.01$) and 4-6 days (CD value = 10.46 at $P > 0.05$) experienced animals in the third test. The persistent preference exhibited by mice exposed at days 4-6 in all the three tests towards the cereal mixture indicates this age to be more sensitive for the establishment of food preferences in *M. booduga*.

4. Discussion

The first series of experiments in the present study establish that foods experienced prior to weaning influence the adult diet choice in *M. booduga* as seen by preferential consumption of such food viz. rice, ragi and wheat compared to a novel food, jowar (table 1). The effect of foods exposed during pre-weaning when for the most part pups do not ingest them is not difficult to explain. After a series of experiments

Table 2. Consumption of familiar and novel foods by *M. booduga* in choice tests.

Batch (N)	Rice I(4)	Ragi II(5)	Wheat III(4)
<i>First choice test</i>			
Earlier ingested food	6.73±0.21	5.96±0.48	6.38±0.15
Jowar (novel)	0.86±0.07	1.39±0.11	1.67±0.21
$P <$	0.001	0.001	0.001
<i>Second choice test</i>			
Earlier ingested food	6.54±0.32	6.17±0.25	7.03±0.46
Jowar (novel)	2.01±0.44	1.88±0.31	1.38±0.14
$P <$	0.001	0.001	0.001

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Structure and function of the truncus arteriosus of the Indian frog *Rana tigerina* (Daud)

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Abstract. This paper presents new results on studies on the Indian frog *Rana tigerina* these include: the presence of a vertical septum in the truncus arteriosus, a cardiac skeleton of cartilaginous nature, a systemic valve in the right systemic arch, and the recording of some events in the cardiac cycle with the help of pressure and electrocardiogram parameters.

Keywords. *Rana tigerina*, truncus arteriosus, electrocardiogram parameters, cardiac cycle

1. Introduction

The lumen of the truncus arteriosus of the frog *Rana tigerina* is divided by a horizontal partition, the septum trunci, into a pulmocutaneous channel and a systemo-carotid channel. A vertical septum in the middle of the truncus further divides the systemo-carotid channel. This tends to separate a stream of less-oxygenated blood from the well-oxygenated blood in the truncus. A systemic valve in the right half of the systemo-carotid channel prevents the entry of less-oxygenated blood into the systemic and carotid arches. The presence of the vertical partition containing chondrioidal tissue in the truncus and a systemic valve and the possible role they played in the distribution of blood, was reported earlier (Nigam 1975, 1980, 1982). This paper describes the detailed structure of the truncus and the flow of blood through it in the light of new findings.

2. Material and methods

Frogs were obtained from the local ponds from July to October. Thirty frogs ranging from 110 mm to 200 mm (mouth to cloaca) were anaesthetized with intra-peritoneal injections of 6 mg/g wt of urethane solution which produced anaesthesia of 2 to 5 hr duration. To observe the gross anatomy of the truncus, it was opened from the ventral side by making a longitudinal slit and carrying it further into the aortic trunks. Truncus was also fixed in Bouin's fluid for microtomy. Following dioxane dehydration paraffin sections of 4 μ thickness were stained via Milligan's trichromic method (Humason 1972). Distribution of blood through the heart and further up into the truncus and arches was observed employing the technique used by de Graaf (1957), (Foxon and Wall 1947; Foxon 1951) and Simons (1959). Small quantities (0.0003 ml) of 1% fluoresceine dye were injected separately and alternately into the right and left atria by micrometer syringe at an interval of 15 min. The course of the injected dye was observed at once under the UV illumination. No frog was injected more than four times in one operation. Simultaneous pressure and

electrocardiogram recordings from various chambers of the heart of the anaesthetized frogs were taken on Polygraph (Grass Co., USA, Model 7).

3. Observations and results

3.1 Heart

The heart of this frog is myogenic and 5-chambered. The atrium is divided into a right and a left chamber by an interatrial septum, and the ventricle is undivided. Since the interatrial septum is shifted more towards the left, the division of the atrium is unequal, the right atrium being double the size of the left. Sinus venosus receives venous blood by three large veins, the vena cavae. Well-oxygenated blood from the lungs is poured into the left atrium by the two pulmonary veins. Conus, the fifth chamber, arises from the right dorsal (Sharma 1957) side of the ventricle and leads into the truncus (ventral aorta). Conus is demarcated from the truncus by an annular sulcus.

Truncus is bifurcated symmetrically into two aortic trunks. After a distance of about 7 mm in a 120 mm long frog, each aortic trunk splits up into a dorsal pulmocutaneous, a middle systemic, and a ventral carotid arch. The conus is supported by cardiac muscles; it has a bore of about 2 mm and is twisted spirally from right to left. The lumen of the conus is separated from that of the ventricle by a set of 4 semilunar ventriculo-conal valves. Another set of similar valves separates the lumen of the conus from that of the truncus. The heart of *R. tigerina* is quite similar to that of the American *R. catesbiana* (Holmes 1975).

Within the conus is a spiral valve (spiral fold of Holmes) hanging as a longitudinal ridge from the dorsal wall of the conus, this divides the lumen of the conus into a right cavum aorticum (systemic channel of Holmes) and a left cavum pulmocutaneum (pulmonary channel of Holmes). Whereas the cavum pulmocutaneum leads into the dorsally placed pulmocutaneous arches by two openings (not one), the cavum aorticum leads into the systemic and carotid arches. The spiral valve is attached to the left wall, but as it extends anteriorly it twists on to the dorsal and then the right wall. At its ventricular end the cavum aorticum is more or less dorsal to the cavum pulmocutaneum, but at its anterior end the positions are reversed.

At the junction of the two aortic trunks (figure 1), is a small vertical septum arising from the dorsal wall of the truncus; the septum is called the septum principale (vertical septum of Holmes). In the middle of the septum principale of *R. tigerina* is a distinct ridge called the septum mediale (Nigam, 1975). The septum principale is shorter than the septum mediale, the latter overtakes the former caudally and terminates freely into a pad-like terminal disc (figures 1, 2, 3). On the left side of the septum principale is the opening of the left systemic arch, on the right there are, besides the opening of the right systemic arch (25 μ across), two openings (each 8 μ across) one leading into the right and the other into the left common carotid arch. Of the three pairs of arterial arches the pulmocutaneous have the largest diameter 75-80 μ , the systemics are 30-40 μ and the common carotids 25-40 μ across.

3.2 The truncus arteriosus

The lumen of the truncus is divided into two unequal channels by a horizontal shelf-like septum trunci (Gaupp 1896) of about 70 μ in thickness. The septum trunci runs

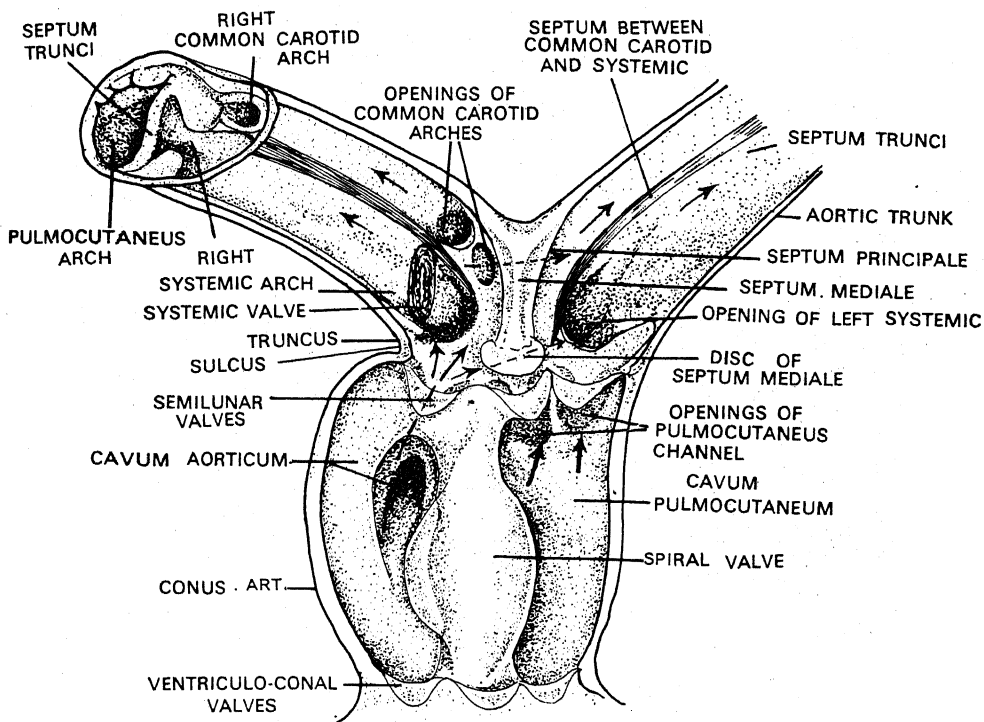


Figure 1. Dissection of the conus and truncus of the frog *Rana tigrina* from the ventral ($\times 20$)

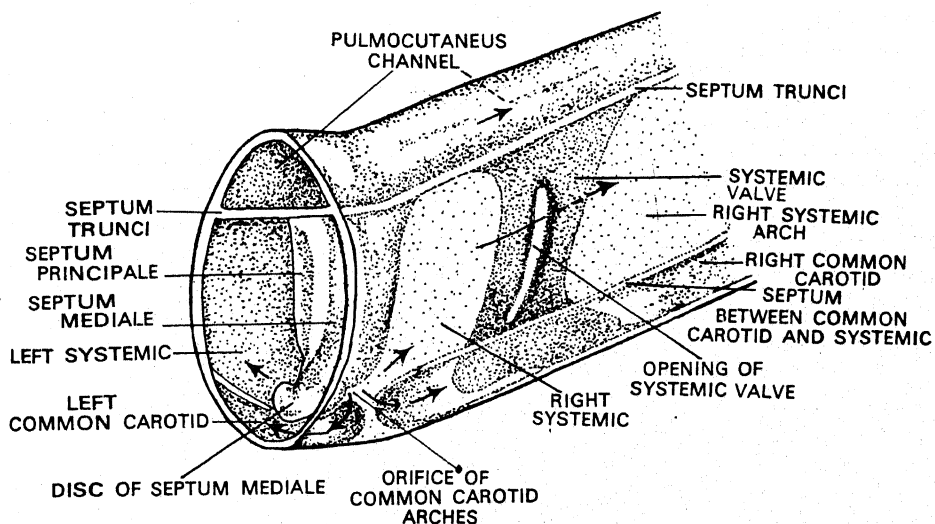


Figure 2. Truncus of frog showing various septa and the systemic valve ($\times 20$).



Figure 3. Microphotograph of the dissection of the truncus of frog showing the septum principale and the terminal disc of the septum mediale (arrows pointing) ($\times 20$).

above the equatorial plane, thus separating a narrow dorsal pulmocutaneous channel from a wider ventral systemo-carotid channel. Pulmocutaneous channel is bifurcated symmetrically into left and right halves (figure 4), the halves are united in the mid-dorsal meridian of the truncus. Each half-channel leads into the pulmocutaneous arch of its side. Septum trunci serves two purposes: (i) it forms the ventral wall of the pulmocutaneous channel and further separates the pulmocutaneous arch from the systemic, (ii) it stands as a divider between the pulmocutaneous channel above and the systemo-carotid channel below.

Unlike the pulmocutaneous channel, the systemo-carotid channel is divided into exact right and left halves by the vertical septum principale. This septum arises from the septum trunci, runs dorsoventrally in the middle of the truncus, and joins the ventral wall of the truncus (figures 4, 5). The dorsal and ventral attached ends of the septum principale are narrow, 75-100 μ wide. The main body of the septum principale is fairly broad, about 400 μ and somewhat dorsoventrally flattened. The septum principale is twisted spirally from left to right in the form of an 'S', the main thrust being asymmetrically more towards the right than towards the left. In the middle of the septum principale is a distinctly visible longitudinal ridge referred to earlier as the septum mediale. For nearly the whole length of it, the septum principale is connected to the ventral wall of the truncus, thus making an effective partition of the systemo-carotid channel dorsoventrally, but towards its caudal end the septum principale falls short of length and terminates before meeting and joining the ventral wall of the truncus. A communicating gap is, therefore, left between the halves of the systemo-carotid channel (figure 6). The septum mediale extends caudally ahead of the septum principale. At this point the septum mediale is flattened and takes the form of a terminal disc, about 1.0 mm across and can be lifted with a needle.

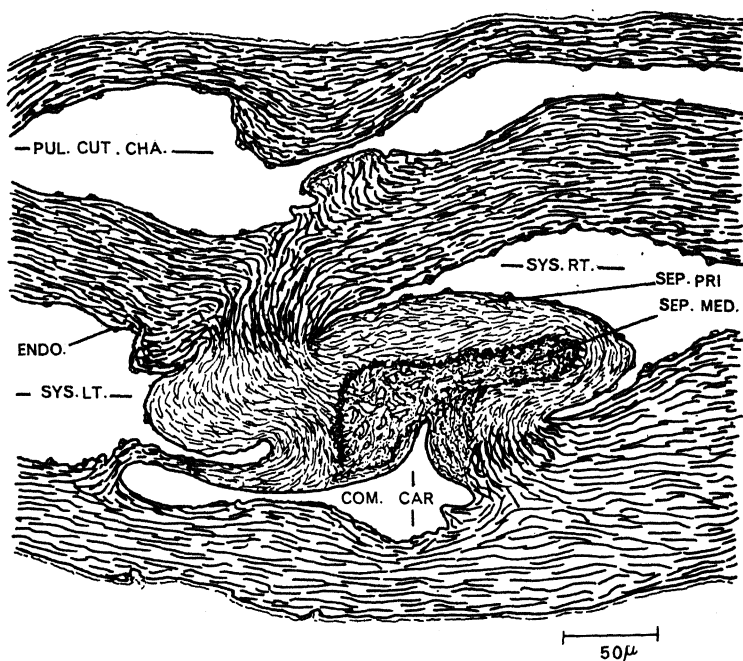


Figure 4. TS through the truncus of *Rana tigerina* Pul. cut. cha—pulmocutaneous channel; sys. rt/lt.—right/left systemic arch; endo.—endothelium; com. car—common carotid orifice; sep. pri.—septum principale; sep. med.—septum mediale.

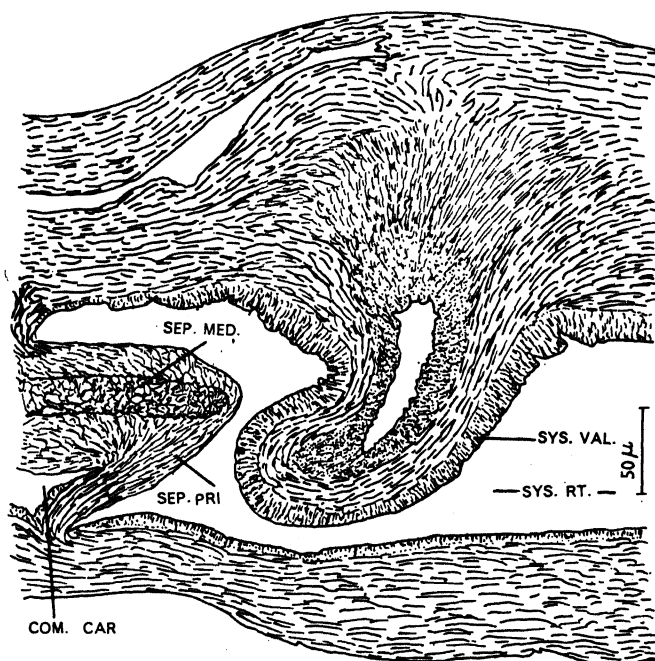


Figure 5. TS truncus through the systemic valve (sys. val.).

3.3 *The systemic valve*

It was first observed in a dissection of the truncus from the dorsal; then confirmed through microtome sections of the truncus. It was named and reported (Nigam 1982). The systemic valve is an obliquely placed, elliptical and muscular ring in the right half of the systemo-carotid channel (figures 1,2,5) very close to the septum principale and proximal to the carotid openings. It arises from the septum trunci and is about $350\ \mu$ away on the right side of the septum principale. Hanging freely from the septum trunci, the systemic valve stands as a 'collapsible door' between the right half of the systemo-carotid channel and the right systemic arch. For about $500\ \mu$ the systemic valve extends anteriorly into the right systemic arch. The base of the valve is about $250\ \mu$ across and about $400\ \mu$ long; it has a longitudinal lumen (figures 2,5). The inclination of the valve is towards the septum principale and after having reached very close to it, the valve joins the ventral wall of the truncus. The valve has a thick core of circular sphincters around its lumen. Between the muscles and the covering endothelium is a thick layer of fibroelastic tissue.

3.4 *Histology of the septa*

The septum principale is largely composed of fibroelastic tissue without muscle cells. The muscles are confined to the origin and insertion of the septum. A thin

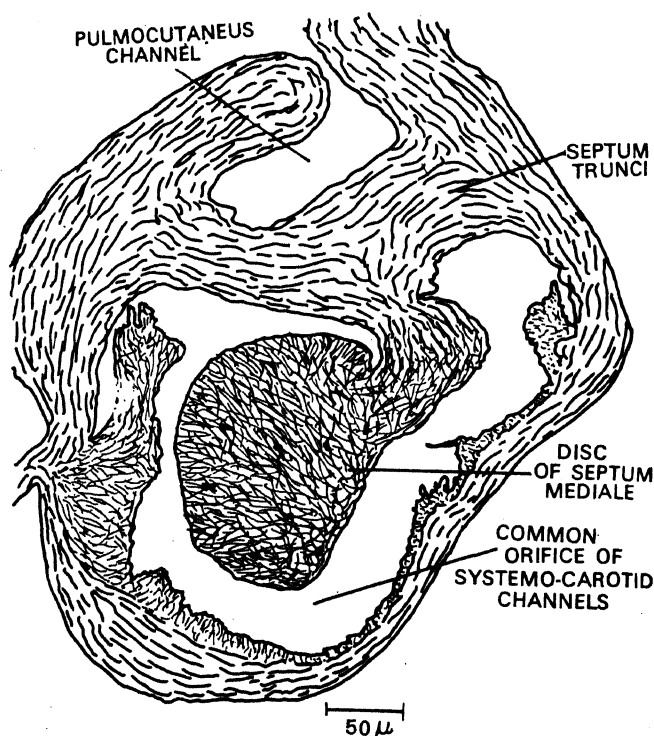


Figure 6. TS truncus through the disc of septum mediale.

sheath of endothelium covers the septum from outside. Septum mediale which is clearly demarcated within the septum principale is composed of densely packed chondrioidal tissue reaching cartilage (Nigam 1980).

3.5 Experimental findings

Injections of fluoresceine dye in the right atrium reveal that with the atrial systole the blood of the right atrium is pumped into the ventricle and occupies roughly 2/3 of the ventricular cavity. Faint branching streaks of the dye penetrating the left 1/3 part of the ventricle indicate some blood received from the right atrium mixing with the blood received into the ventricle from the left atrium. Ventricular systole sends most of the blood of the right side up into the conus on one side of the spiral valve and fills the cavum pulmocutaneum fully.

Injections of the same dye in the left atrium reveal that the left atrium contracts about 0.03 sec after the contraction of the right atrium. Blood of the left atrium is received in the left 1/3 part of the ventricular cavity. Upon ventricular systole this blood and also some from the right atrium received earlier passes into the carotid and systemic arches *via* the systemo-carotid channel.

Simultaneous pressure and electrocardiogram (ECG) recordings (figure 7) of sinus venosus, right/left atrium, ventricle and the conus (Nigam and Shrivastava 1981) have produced a complete picture of the events taking place in the cardiac cycle of 0.94 sec in this frog. In addition to the conventional P, Q, R, S and T waves in the ECG, a V-wave representing the activity of the sinus venosus has been observed in the frog. These recordings have further established that (a) systole of the left

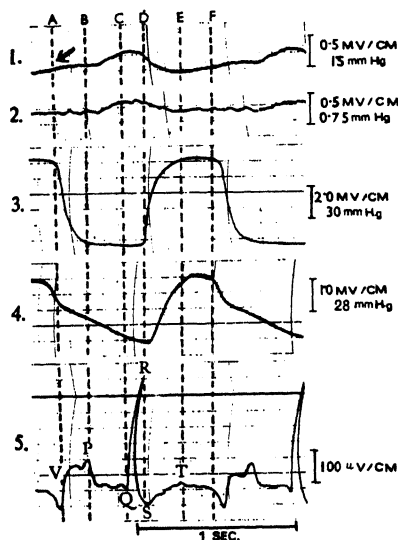


Figure 7. Pressure and electrocardiogram recordings of the right atrium-1, left atrium-2, ventricle-3, conus-4, aligned with the ECG-5 Lead II recordings. A—beginning of V-wave marked by an arrow; B—opening of AV valve; B-C atrial systole; C—closure of AV valve and beginning of ventricular contraction and opening of ventriculoconal valves; D—beginning of ventricular and conal systole.

atrium is delayed by 0.03 sec over that of the right atrium, and (b) the systole of the ventricle and the conus is in two phases, a phase of maximal ejection, followed by a phase of reduced ejection.

In the first phase of the ventricular systole most of the blood of the right side of the ventricle is pumped into the cavum pulmocutaneum. The cavum aorticum receives the remaining blood in the reduced ejection phase of the ventricle. This is followed by the conal systole almost synchronically with the ventricle, but with a short lapse of about 0.04 sec. In the first phase of conal systole the less-oxygenated blood of the cavum pulmocutaneum passes into the pulmocutaneous channel and thence into the pulmocutaneous arches. No blood from the cavum aorticum passes into the other two arterial arches during the phase of maximal ejection of the conus because of two reasons (a) the sphincters of the systemic valve keep its lumen closed thereby blocking the entry of blood into the right systemic arch, and (b) while the blood is passing through the pulmocutaneous channel during the first phase of conal systole, the latter is inflated and depresses the septum trunci which in turn pushes the septum mediale down, its terminal disc reaching the ventral wall of the truncus and blocking the entry of blood into the orifice of the common carotids leading into the carotids and left systemic arch. It is only when the less-oxygenated blood of pulmocutaneous channel has passed over into the pulmocutaneous arches, the above restraints are lifted and the blood of the cavum aorticum (containing well oxygenated and some mixed blood) is allowed to pass into the systemic and carotid arches.

4. Conclusions

Although Sharma (1957) had studied the structure and mode of working of the heart of *Rana tigrina*, he did not find structures such as the septum trunci, septum principale, septum mediale and the systemic valve. He also did not find the presence of a cardiac skeleton in the heart of frog which helps to strengthen the septum principale in making an effective partition of the truncus. The said structures have now been found to play a great role in the distribution of blood through the truncus. Pressure and ECG recordings have, for the first time, enabled working out the complete cardiac cycle in this frog.

Acknowledgements

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* The spelling of *Rana tigrina* has been changed to *Rana tigerina*.

Effect of solar eclipse on the emergence of some freshwater cercariae

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Abstract. The effect of solar eclipse on the emergence of five species of freshwater cercariae from their snail hosts has been studied. Two cercariae showed visible changes from their normal emergence pattern from the snails during eclipse. It appears that changes in atmospheric light and temperature brought about by eclipse altered the behaviour of the snail and this has affected the emergence of cercariae.

Keywords. Cercariae; solar eclipse; *Cercariae indicae*; cercarial emergence.

1. Introduction

On 16 February 1980 (Saturday) total solar eclipse occurred for 2 to 5 minutes in a belt of 135 km from 14.17 hrs to 17.00 hr across peninsular India. Visakhapatnam, situated outside this belt, witnessed 95% eclipse for 2 minutes from 15.46 to 15.48 hr when the temperature dropped by 2°C. The eclipse commenced at 14.30 hr and terminated at 16.58 hr. Visible changes in light and temperature were observed between 15.30 hr and 16.30 hr.

Since cercarial emergence from snails is mainly controlled by light and temperature a simple experiment was designed to study if the climatic changes brought about by the solar eclipse alter the normal emergence pattern of cercariae. Five different cercariae showing different rhythms in their emergence from snails were selected for study. The hourly changes in the numbers of cercariae emerging from snails were followed for three consecutive days, the eclipse day and the pre- and post-eclipse days. The results for the three days are compared and the observed variations discussed in relation to changes in climatic factors brought about by the eclipse.

2. Materials and methods

Snails were obtained from two different sources: (i) 186 specimens of *Indoplanorbis exustus* collected from a lake at Kondakarla (ii) 45 individuals of *Thiara tuberculata* collected from a stream at Waltair. The infected snails were spotted out and the following 5 species of cercariae were selected for study.

2.1 *Cercariae indicae* VIII Sewell, 1922

A pleurolophocercous cercaria belonging to Opisthorchioidea collected from *Thiara tuberculata*. The cercaria is fairly big in size, a swift swimmer and is uniformly distributed throughout the water column. It did not exhibit any photoperiodism

although equipped with eyespots.

2.2 *Cercariae indicae* XVII Sewell, 1922

A medium sized xiphidiocercaria of polyadenous group belonging to Plagiorchioidea emerging from the snail *Indoplanorbis exustus*. The cercaria swims close to the bottom of the dish and is thus positively geotropic.

2.3 *Cercariae indicae* V Sewell, 1922

A very small xiphidiocercaria from *T. tuberculata* identified as a plagiorchioid cercaria. The cercariae are negatively geotropic and they accumulate in large numbers close to the surface of water column forming a thick suspension of cercariae.

2.4 *Echinostome cercaria*

An unidentified 27 spined echinostome cercaria from the snail *I. exustus*. The cercaria is fairly big in size appearing as a white speck moving actively in the water. It did not exhibit phototropism or geotropism.

2.5 *Strigeid cercaria*

An unidentified strigeid cercaria with an inflated tail and two short furcae, emitted by *I. exustus*. The cercariae remain suspended in water at all levels.

The infected snails were placed individually in test tubes containing stored tap water and the test tubes were placed in an open space on the terrace of a two storied building where the temperature and light effects are easily felt. The numbers of cercariae that had emerged from the snail were counted at hourly intervals for three consecutive days, the eclipse day and pre- and post-eclipse days. The experiment began at 1000 hr on 15 February and ended on 18 February at 1000 hr. This covered the eclipse period which fell between 1530 and 1630 hr on Saturday, 16 February. When the cercariae were to be counted, the snails were removed to a new test tube and a small amount of 5% formaldehyde was added to the water in the used test tube in order to kill the cercariae. The cercariae that had settled down to the bottom of the test tube were counted. Snails were not fed during the experiment.

The significance of the differences in the numbers of cercariae that had emerged during eclipse time and during the same time on pre-eclipse and post-eclipse days were tested statistically by χ^2 test wherever possible.

3. Observations

3.1 *Cercariae indicae* VIII

The periodicity in the emergence of the cercaria from the snail for the 3 days is shown in figure 1. Cercariae emerged from the snail throughout the day but the peak emergence occurred between 1600 and 1800 hr. The cercarial emergence on the eclipse day followed the same periodicity as on the other two days but a notable feature is that prior to the commencement of eclipse *i.e.* during the period between 1400 and 1500 hr on 16 February, because of intense heat the snail became inactive and as a result no cercariae were shed during this period. After the commencement of eclipse and associated sudden drop in temperature and light intensity, the snail regained activity and significantly more cercariae were shed during the eclipse

(between 1600 and 1700 hr than during the same period on the other days ($P < 0.05$). Thus there seems to be some enhancement in the shedding of cercariae from the snail during the eclipse period. On the third day of the experiment there is some depletion in the numbers of cercariae emerging from the snail.

3.2 *Cercariae indiciae XVII*

This cercaria emerged predominantly during darkness between 1900 and 0800 hr the peak being at 2400 hr. No cercariae emerged during day time between 0900 and 1800 hr (figure 2). During eclipse, however, some cercariae emerged between 1600 and 1800 hr.

3.3 *Cercariae indiciae V*

This cercaria emerged in large numbers from 1600 hr to 0400 hr the peak emergence being between 0500 and 1500 hr. Similar rhythm was observed on all the three days but on the first day of the experiment, cercariae showed two peaks in their emergence from the snail, one at 2000 hr and another at 2400 hr. There was considerable depletion in the numbers of cercariae that had emerged from the snail on the third day of the experiment (figure 3).

3.4 *Strigeid cercaria*

The emergence of this cercaria appears to be stimulated by light as it emerged between 1000 hr and 2000 hr when high light intensity conditions prevailed (figure 4). Shedding was most intense during bright daylight. Some difference was observed

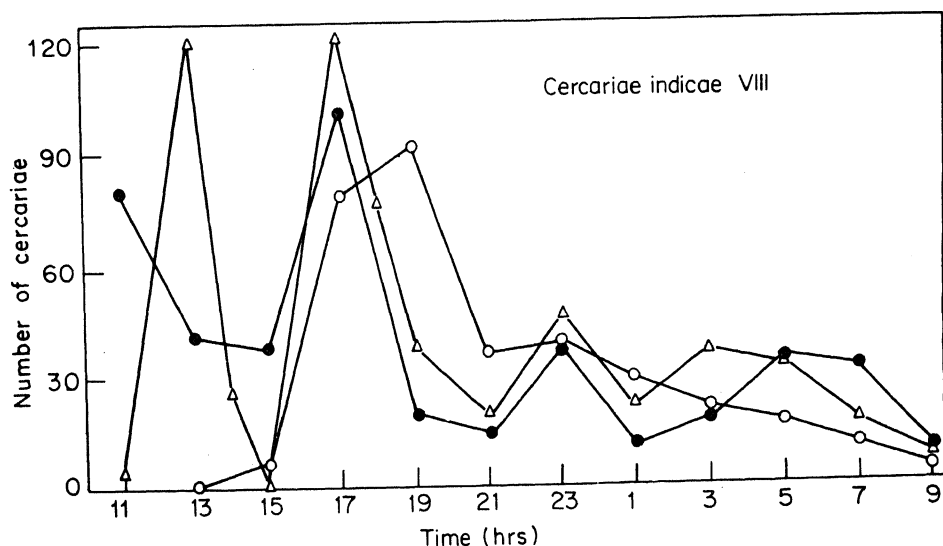
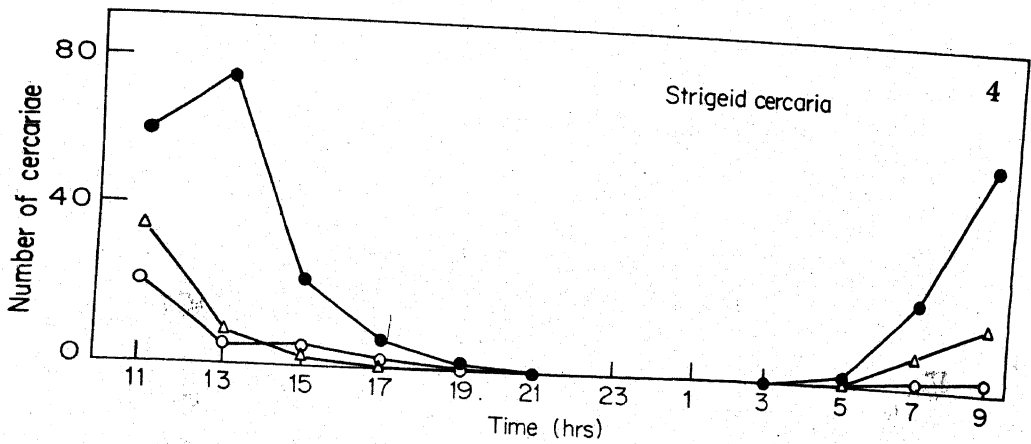
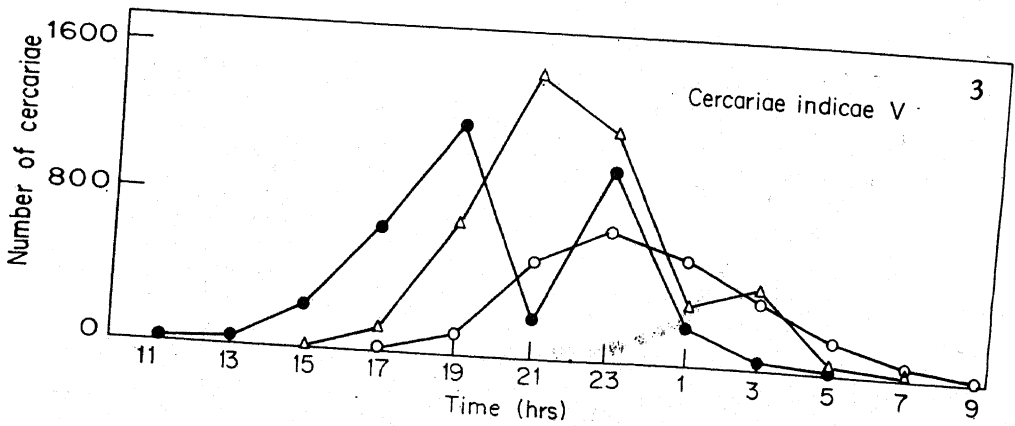
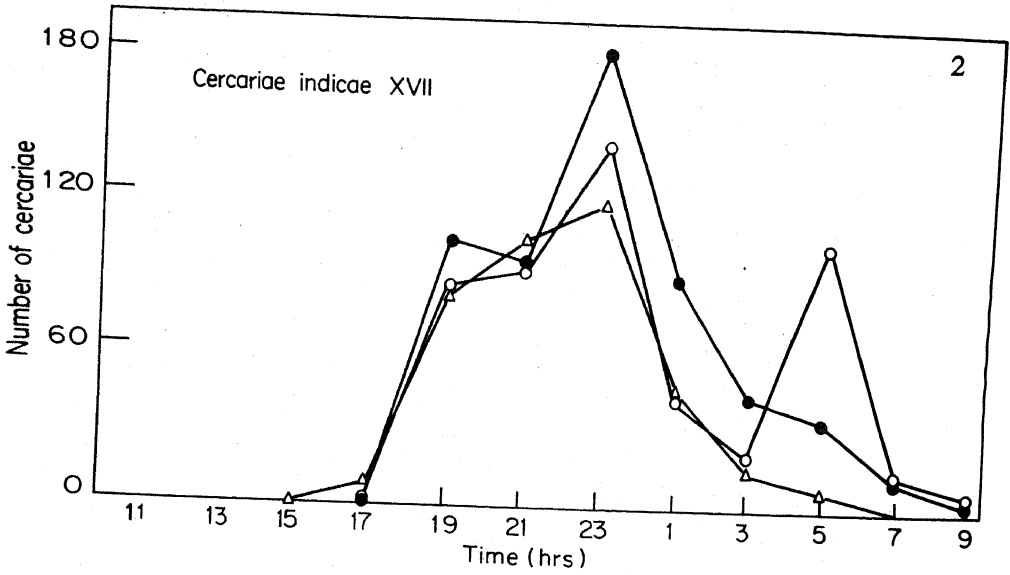
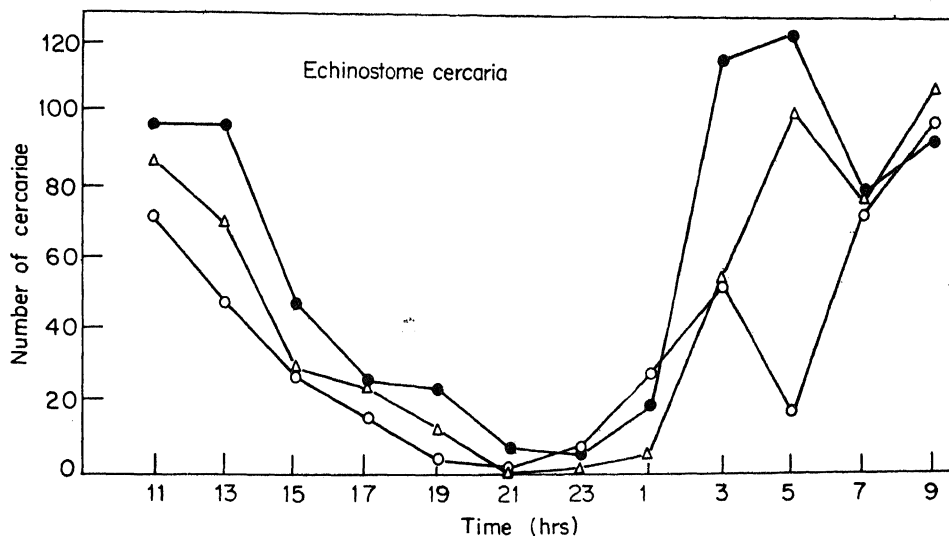


Figure 1. See caption in p. 69.



Figures 2-4. See captions in p.



Figures 1 to 5. Periodicity in the emergence of cercariae for 3 days. Each point on the graph indicates the numbers of cercariae that had emerged during the 1 hr period prior to the time indicated in the abscissa. ● Emergence from 1000 hr on 15 February to 0900 hr on 16 February; Δ from 1000 hr on 16 February to 0900 hr on 17 February; O, from 1000 hr on 17 February to 0900 hr on 18 February.

in the cercarial emergence on the eclipse day. In the normal days cercariae continued to emerge till 2000 hr but on the eclipse day the emergence of cercariae ceased right from 1600 hr and this may be a reflection of the drop in the light intensity during eclipse time. On the third day of the experiment the snail host became inactive, hence only a few cercariae were shed.

3.5 *Echinostome cercaria*

This cercaria emerged throughout the day but only in small numbers between 2000 hr and 0200 hr, the peak emergence being between 0400 and 600 hr (figure 5). No changes were observed in the emergence pattern of the cercaria during eclipse time. The slight variations noted were not statistically significant.

4. Discussion

Among the free swimming larval stages of helminths, cercariae are known to exhibit rhythms in their emergence from the molluscan hosts. Some cercariae emerge maximally during day-time, others at night and there are some which do not show any periodicity in their emergence pattern. Previous studies on the emergence of cercariae from snails and the influence of various environmental factors on the cercarial emergence suggested photoperiod and thermoperiod as the major synchronising factors controlling the process (Rees 1931; Kuntz 1947; Olivier 1951; Asch 1972; Wagenbach and Alldredge 1974; Combes and Theron 1977). Other factors such as pH and oxygen content of the medium, nutritional condition and physiological state of the host also exert their influence either individually or in

combination with others (Mohandas 1974). The peak period of emergence of cercariae from the snail is also correlated to the period of peak occurrence of hosts to which the cercariae attack (Yamaguti 1970)

Of the five cercariae subjected to experimental work in the present study, the strigeid cercaria emerged only during day-time and in large numbers under bright light, thus the emergence of this cercaria seems to be elicited by light. Among others, *Cercariae Indicae* V and XVII emerged only during periods of darkness in the absence of light. Thus they reacted negatively to light. *C. Indicae* VIII and the echinostome cercaria emerged regularly at all hours of the day and night although more were shed in daylight than in diffuse light, or at night. Light does not seem to be the causative factor for the emergence of these cercariae.

Only two cercariae responded to climatic changes brought about by eclipse and showed some deviations from their normal pattern of emergence. The strigeid cercaria, the emergence of which is triggered by light, stopped emerging during eclipse time and this appears to be directly related to the disturbance in light intensity caused by solar eclipse. Similarly *C. Indicae* XVII which emerges only during dusk and night and not during daytime, emerged though in small numbers during eclipse as a result of fall in temperature and light. Among others, *C. Indicae* VIII showed some variation from the normal periodicity in that it emerged in significantly greater numbers during eclipse period than during the same period on the other days. This enhancement in the numbers of cercariae that had emerged appears to be a result of the change in the behaviour of snail which became active following the fall in temperature and light during eclipse time.

In three of the five cercariae studied, there is some decline in the numbers of cercariae released on the third day of the experiment. Since the snails were not fed during the experimental period, the decline may have been due to nutritional factors.

It is evident that eclipse did influence the periodicity of some cercariae. However, this influence appears to be only of an indirect nature. The change in atmospheric light and temperature during eclipse period altered the behaviour of the snail which resulted in changing the cercarial emergence pattern. As stated by Erasmus (1972) the presence of larval trematodes in a mollusc represents a particularly close host-parasite relationship with variations in the physiology of the one having profound effects on the other.

Acknowledgement

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Studies on the meiofauna of Sagar Island

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Abstract. Mean number of total meiofauna individuals in the intertidal estuarine sediments around the island ranged from $1/10 \text{ cm}^2$ to $517/10 \text{ cm}^2$. Nematodes and copepods were the dominant taxa comprising over 80% of the total fauna. Higher meiofaunal densities occurred over the lower shore. The fauna was concentrated mostly in top 4 cm of sediment, showing little submergence towards higher tidal levels. No diurnal and seasonal vertical migrations of meiofauna were observed. Two small seasonal peaks of meiofaunal abundance were noted. Total population values were highest in summer and lowest during monsoon. Nematodes reached their maximum in winter and copepods in summer. Variations in salinity appeared to be responsible for the seasonal pattern. The present meiofaunal densities are considerably lower than those recorded in other areas. The highly fluctuating hydrographical conditions and the fine sediment with silt-clay fraction, poor drainage, low oxygen content, etc., are considered responsible for the paucity of meiofauna in this area.

Keywords. Meiofauna; composition; density; Sagar Island.

1. Introduction

The interest in a study of the distribution and ecology of meiofauna in sediments of fluctuating salinity has been steadily increasing in recent years. Compared to marine habitats, the distribution of estuarine meiofauna has been investigated only in a few geographically isolated areas of the world (Coull 1973). Very little is known about it in the Indian waters, the studies of McIntyre (1968), Patnaik (1971), Damodaran (1974), Sarma and Ganapati (1975) and Sarma and Rao (1980) being notable in this regard. Hitherto, nothing is known of the meiofauna inhabiting the Gangetic estuarine system, the largest of its kind on the Indian coast. Hence, the present investigation was undertaken to make a preliminary survey of the composition, density and distribution of meiofauna within the intertidal deposits around Sagar Island, located at the Gangetic mouths. This paper deals with the results of the study carried out for 2 years from April 1978 to March 1980.

2. Material and methods

2.1 *Area investigated*

Sagar Island, the western most part of Sunderbans in the Bay of Bengal, is situated 96 km south of Calcutta, between latitudes $21^{\circ}37'$ and $21^{\circ}52'$ N and longitudes $88^{\circ}03'$ and $88^{\circ}11'E$. The island is somewhat triangular in outline, about 30 km in length, 16 km in maximum width and 380 sq km in area. It is flanked by two rivers, Hooghly on the west and Baratala on the east. On the southern side, it faces the open sea. It is a flat delta with many tidal creeks and muddy shores on either side

and fine sandy beaches on the southern side. The soil is mostly of river borne silt, sometimes with overlying sand. Three seasons are well experienced in this region, the summer from March to June with high temperature, the monsoon from July to October with heavy rainfall and the winter from November to February with low temperature. The annual cycle of water temperature, salinity and plankton in the Hooghly estuarine system was given by Saha *et al* (1975). After a preliminary survey of the coast around the island, 6 areas were selected for the present investigation (figure 1), of which the site at Gangasagar (station 6) was chosen for a detailed study of the fauna.

Gangasagar is located about half-way on the south coast. Here, the sea is shallow and the beach is moderately a sheltered one, with an extensively wide stretch of intertidal sand flat exposed at low tide. A horizontal distance of about 240 m was exposed during low water spring tides. The other 5 subsidiary areas located on river bank are in the vicinity of Radhakrishnapur, Mandirtala, Kachuberia, Kaylapara and Chemaguri. In these places, extensive mud-flats of varying width are exposed at low tide. The sediment is of uniformly soft mud, with high organic content.

2.2 Techniques of study

All the field observations and collections were made during low tide. Environmental parameters of the beach were studied only at Gangasagar. An intertidal transect

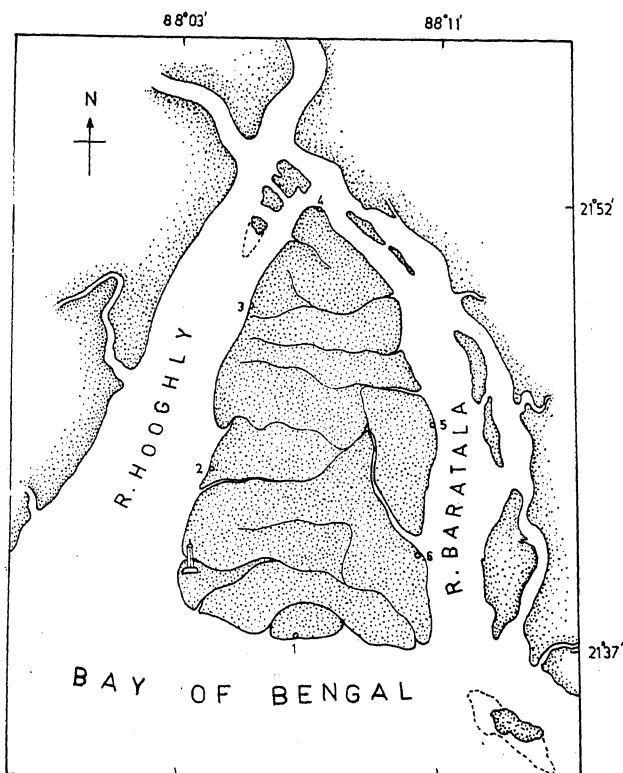


Figure 1. Sagar Island, showing the study areas 1-6. 1. Gangasagar; 2. Radhakrishnapur; 3. Mandirtala; 4. Kachuberia; 5. Kaylapara; 6. Chemaguri.

was established on the beach with 4 sampling stations, A-D, between MLWS and MHWS (figure 2). Beach profiles were obtained by using the survey technique of Emery (1961). For granulometric analyses, core samples of the sediment were collected from the top (10 cm) at 4 stations along the transect. The amount of capillary water present at different levels of the beach was determined by weighing the wet sand samples and reweighing the same to constant weight after they were completely dehydrated. The temperature of adjacent sea water was recorded in knee deep water with a sensitive centigrade thermometer and that of the intertidal sediment by inserting the thermometer to depths of 1, 5, 10, 15 and 20 cm below surface at all the 4 stations. Pore water samples were collected with a hypodermic syringe following the method of Johnson (1967). Salinity determinations were made by Knudsen's method, while Winkler's method was used in estimating the amount of dissolved oxygen. Particulate organic matter of the sediment was approximately determined by the loss in weight on ignition.

For a quantitative study, regular monthly sampling was conducted at Gangasagar during lowest spring tides. Due to difficulties in sampling and sieving, collections at the 5 muddy areas were made only at one point on the traverse between low and midwater levels. Sediment samples were taken using a hand-operated metallic corer, 30 cm in length and 20 cm² in internal cross sectional area. The corer was longitudinally cut into two halves and hinged on one side, so that it could be easily opened without disturbing the sediment core. The core samples were cut into 2 cm thick segments to ascertain the vertical distribution of meiofauna and anaesthetized with a solution of MgCl₂ isotonic with sea water. Animals were extracted from these samples by decanting and sieving method. The fauna passing through a 1 mm sieve and retained on a 45 μ m sieve are considered as meiofauna in the present investigation. Faunal densities are expressed as the number of individuals per 10 cm² and each value represents the mean of two cores.

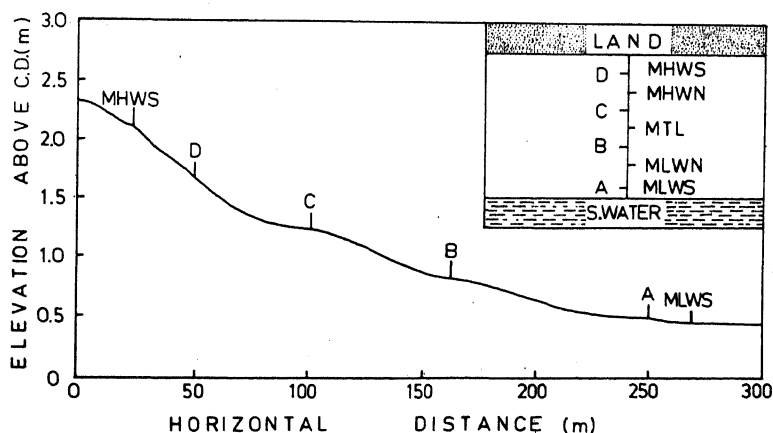


Figure 2. Selected beach profile at Gangasagar (18 November 1979), showing the position of sampling stations A-D along the intertidal transect.

3. Results

3.1 *The environment*

The beach slope at Gangasagar was uniformly very low and a typical profile recorded is shown in figure 2. The sand was largely fine in texture with a considerable percentage of silt or clay, which generally increased with depth in the sediment (figure 3). The beach was fully saturated nearly up to station C, resulting in a relatively permanent ground water-table close to the surface. Temperature values of the intertidal sediment followed the usual pattern of summer maximum and winter minimum, with variations mostly occurring in the upper 5 cm of the sediment towards higher tidal levels (figure 4). The salinity values of interstitial water in the beach were generally the same as that of the adjacent sea water (figure 5). The concentration of dissolved oxygen in the beach showed a distinct decrease with distance from low water level and particularly with depth in the sediment. The beach sands were sufficiently rich in organic detritus.

3.2 *Composition of meiofauna*

Free-living nematodes and harpacticoid copepods constituted the major groups of meiofauna in this area. Turbellarians, polychaetes, oligochaetes, ostracods, isopods, amphipods, cumaceans and halacarids occurred in small numbers. Hydrozoans and nemerteans were rare. The larval forms of polychaetes, crustaceans, molluscs and insects were present. The mean annual density and percentage abundance of the diverse groups of meiofauna at the most frequently sampled station B during the period of study are given in table 1. True interstitial forms were rare, while the burrowing ones predominated in the habitat.

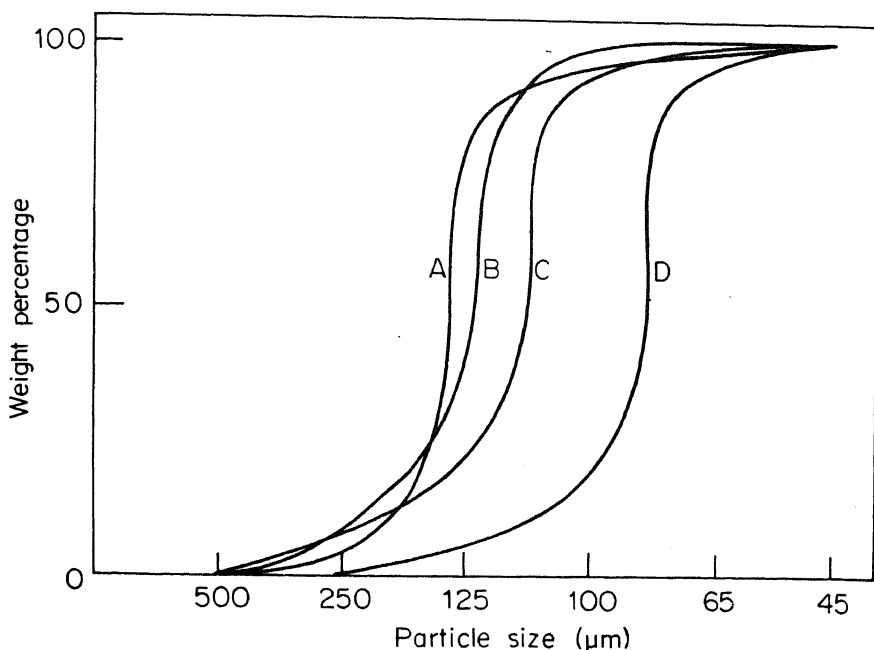


Figure 3. Representative cumulative curves of sediment particle size for the 4 intertidal stations A-D.

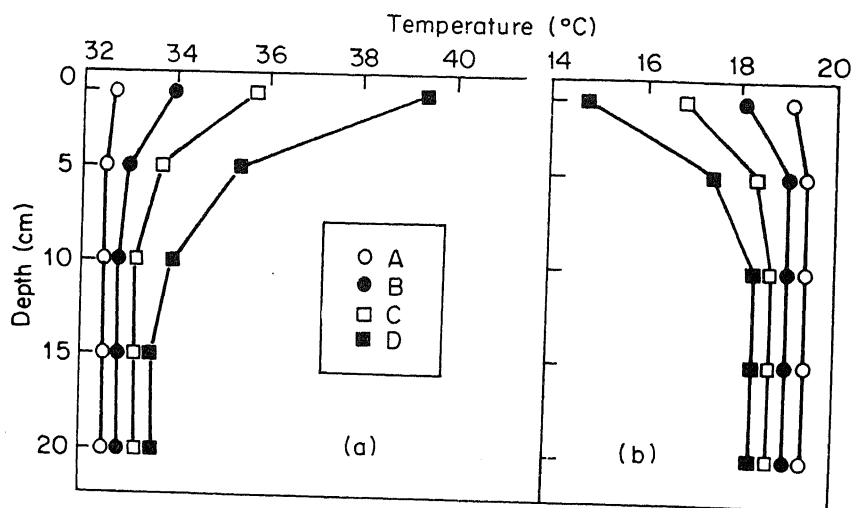


Figure 4. Temperature gradients in the sediment at the 4 intertidal stations. a — Summer (14 May 1979); b — Winter (28 December 1979).

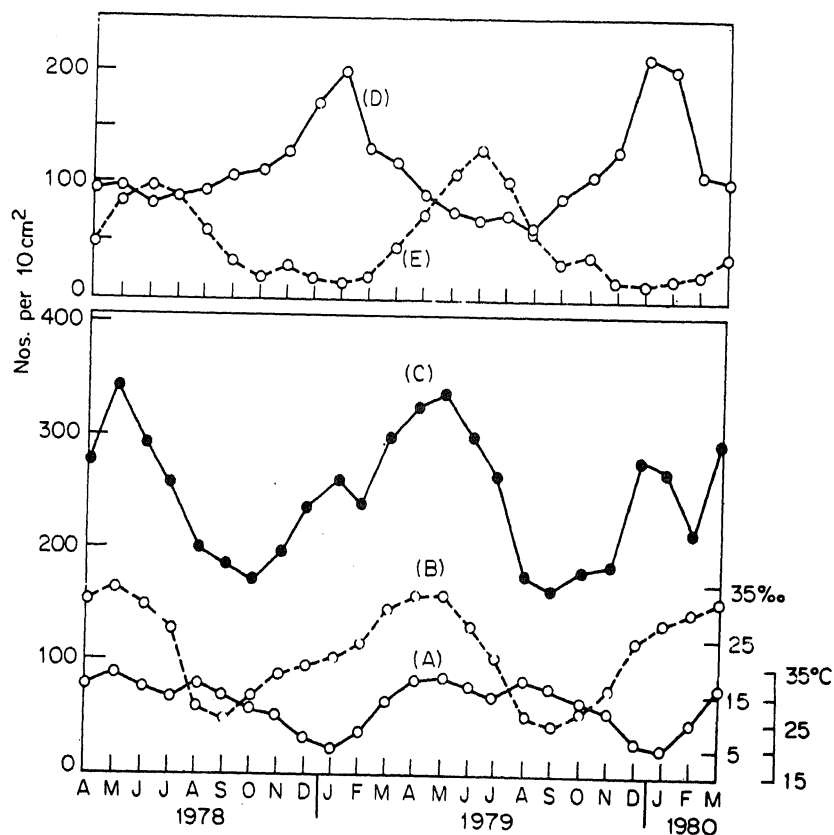


Figure 5. Comparison of seasonal changes in temperature and salinity of sea water with the meiofaunal abundance at station B. A—Temperature; B—Salinity; C—Total meiofauna; D—Nematoda; E—Copepoda.

3.3 Horizontal distribution

The entire intertidal transect was sampled to a depth of 30 cm thrice in a year during summer (May), monsoon (September) and winter (January) to study the horizontal and vertical distribution pattern of the meiofaunal populations against fluctuating temperature and salinity regime in the habitat. No remarkable variations were detected during these periods in their spatial distribution within the beach, indicating that the fauna occupied the same horizontal and vertical levels in the sediment throughout the year. The mean values of population densities at different levels based on the six complete transects are presented in table 2. As the core samples collected 10 cm below the sediment surface did not contain any animals, the present data are limited to that depth. Distribution of the total population indicated that bulk of the meiofauna was concentrated on the lower half of the beach, showing a general decrease towards high water level. Of the total fauna collected at the 4 intertidal stations, 28.81% of them occurred at station A, 43.74% at station B, 19.15% at station C and 8.30% at station D. The density and diversity of meiofaunal groups showed a distinct decrease towards higher tidal levels.

3.4 Vertical distribution

No distinct seasonal or diurnal vertical migrations of the meiofauna were observed during the study period and the total populations were close to the surface throughout the year (table 2). Of the total fauna collected at the 4 intertidal stations, 63.93% of them occurred in the upper 2 cm of the sediment, 25.30% between 2-4 cm, 7.02% between 4-6 cm, 3.03% between 6-8 cm and 0.72% between 8-10 cm depth. Between low and half-tide levels, the fauna occurred mostly in the top 4 cm, while it was found at 2-6 cm below the surface towards higher tidal levels. Thus, little meiofaunal submergence occurred towards higher levels due to reduced water content in the upper layers of the sediment.

3.5 Seasonal distribution

Quantitative studies on meiofaunal population density during different months of the year were carried out at station B and the results are presented in table 3. The

Table 1. Composition of meiofauna (nos./10 cm²) at station B during April 1978 to March 1980.

Group	Maximum	Minimum	Mean	Percentage
Turbellaria	17	0	6.50	2.65
Nematoda	218	66	117.17	47.84
Polychaeta	21	0	9.42	3.85
Oligochaeta	7	0	1.75	0.71
Ostracoda	11	3	6.79	2.77
Copepoda	134	16	51.87	21.18
Nauplii	97	0	33.96	13.87
Isopoda	11	0	3.37	1.38
Amphipoda	5	0	2.04	0.83
Others	21	5	12.04	4.83
Total meiofauna	342	160	244.91	

Table 2. Mean population densities (nos./10cm²) of meiofauna in the upper 10 cm of the sediment at the 4 intertidal stations, A-D.

Meiofauna group	Depth (cm)	A	B	C	D
Turbellaria	0-2	7	11	5	0
	2-4	1	2	2	1
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Nematoda	0-2	124	192	47	14
	2-4	37	64	78	36
	4-6	13	19	21	27
	6-8	7	11	14	6
	8-10	2	3	3	1
Polychaeta	0-2	16	21	2	0
	2-4	2	4	5	1
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Oligochaeta	0-2	1	2	4	3
	2-4	0	2	6	7
	4-6	0	0	1	2
	6-8	0	0	0	0
	8-10	0	0	0	0
Ostracoda	0-2	9	13	4	0
	2-4	0	0	1	0
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Copepoda	0-2	69	93	18	0
	2-4	7	11	9	0
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Nauplii	0-2	38	57	5	0
	2-4	3	8	2	0
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Isopoda	0-2	3	5	1	0
	2-4	1	2	2	1
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Amphipoda	0-2	2	3	1	0
	2-4	1	1	1	0
	4-6	0	0	0	0
	6-8	0	0	0	0
	8-10	0	0	0	0
Others	0-2	12	15	3	1
	2-4	5	7	5	3
	4-6	1	2	1	1
	6-8	0	0	0	0
	8-10	0	0	0	0
Total meiofauna	0-2	281	412	90	18
	2-4	57	101	110	49
	4-6	14	21	23	30
	6-8	7	11	14	6
	8-10	2	3	3	1
	Totals	361	548	240	104

Table 3. Seasonal abundance (nos./10 cm²) in the monthly composition of meiofauna at station B during April 1978 to March 1980.

Months	Turbellaria	Nematoda	Polychaeta	Oligochaeta	Ostracoda	Copepoda	Nauplii	Isopoda	Amphipoda	Others	Total meiofauna
1978 April	13	94	16	2	6	47	74	4	2	13	271
May	17	98	21	0	9	82	93	7	4	11	342
June	9	82	17	0	11	96	51	5	4	9	284
July	2	87	18	1	8	87	37	4	2	7	253
August	0	93	12	4	6	58	14	2	1	10	200
September	5	107	14	3	4	31	4	0	0	11	179
October	1	114	11	5	7	19	0	0	2	5	164
November	12	127	2	1	6	27	2	2	3	12	194
December	3	172	0	0	10	18	6	3	0	18	230
1979 January	0	201	2	0	7	16	11	2	1	14	254
February	7	134	5	2	6	21	36	0	3	18	232
March	11	120	13	1	9	43	81	4	2	11	295
April	14	93	18	0	4	76	97	6	4	15	327
May	12	81	17	2	6	112	74	11	4	17	336
June	3	74	11	1	6	134	42	7	5	13	296
July	3	78	13	0	9	107	27	5	1	19	262
August	2	66	6	3	4	65	18	1	0	10	175
September	0	92	9	7	3	36	5	0	2	6	160
1979 October	2	106	4	4	8	41	2	3	1	7	178
November	7	135	0	1	7	20	0	4	0	9	183
December	9	218	1	1	5	17	14	0	0	12	277
1980 January	5	211	2	2	6	24	9	2	2	8	271
February	8	115	5	0	9	27	35	3	3	13	218
March	11	114	9	2	7	41	83	6	3	21	297
Mean	6.50	117.17	9.42	1.75	6.79	51.87	33.96	3.37	2.04	12.04	244.91

seasonal fluctuations indicated two small peaks of meiofaunal abundance in a year, a major one during summer and a minor one in winter. Lowest densities of total populations were recorded during and immediately after the monsoon months. As more than 80% of the total fauna comprised of nematodes and copepods, fluctuations in total meiofauna numbers mostly depended on the abundance of these two groups. The maximum copepod production during summer months was mainly responsible for the major peak in meiofauna numbers, while the nematode increase in winter for the minor peak. The nematodes formed a relatively constant proportion in meiofauna population with little seasonal fluctuation in their numbers, while the monsoon months were the poorest for copepod production. The other groups of meiofauna did not indicate any distinct pattern in their seasonal abundance. During the 2 years of study, the peak periods and the low levels of abundance nearly remained the same for major meiofaunal groups.

3.6 *Meiofauna of the muddy beaches*

The mean density values of meiofauna individuals in the fine intertidal estuarine muds recorded at the 5 subsidiary study areas are presented in table 4. The vertical distribution of meiofauna at all these sites conformed to the usual pattern, bulk of the total fauna being confined to the upper 1 cm of the sediment. Free-living nematodes formed the dominant element, averaging about 80% of the total meiofauna numbers. Copepods were the next abundant group. Abundance of meiofauna individuals and poverty of species appeared to prevail in these areas. Further, the density and diversity of meiofauna in these muds indicated a general decrease from sea towards the upper reaches of the estuarine system. As no regular monthly sampling was carried out at these areas, nothing can be said about the seasonal abundance of meiofauna.

4. Discussion

In many of the world beaches studied, the nematodes formed a relatively stable and dominant element of the meiofauna due to their ability to withstand fluctuating conditions in the habitat, while copepods were generally second in overall abundance (McIntyre 1968, 1969; Harris 1972; Coull 1973; Martinez 1975; Platt 1977; Dye and Furstenberg 1978; Moore 1979). The present investigation also

Table 4. Mean numbers of meiofauna individuals per 10 cm² at the 5 muddy areas 2-6 on Sagar Island (vide figure 1) during March 1979 and 1980.

Areas	2	3	4	5	6
Nematoda	306	228	65	249	412
Copepoda	32	18	3	12	23
Ostracoda	21	16	0	5	14
Turbellaria	13	14	2	7	17
Polychaeta	14	8	2	11	21
Isopoda	11	3	0	0	7
Amphipoda	3	0	0	1	2
Others	13	17	4	13	21
Totals	413	304	76	298	517

indicated a nearly similar composition, the nematodes predominating in all the areas studied on the island, the finer muddy sediments supporting slightly higher populations over the sandy ones. Compared to the mud deposits, the copepods are well represented in sandy areas (McIntyre 1969). The numerical abundance of the remaining smaller groups of meiofauna reported from different global regions varied from area to area, making their comparison difficult. The blocking of interstices in these fine sands with silt-clay fraction has practically eliminated many of the truly interstitial species and groups characteristic of the habitat. As bulk of the beach sands fall under $160\text{ }\mu\text{m}$ in mean particle size, the meiofauna of this area is to be considered mostly of the burrowing type, excepting the nematodes capable of sliding even in very small spaces (McIntyre and Murison 1973; McLachlan *et al* 1977; Dye and Furstenberg 1978; Moore 1979).

Due to the presence of a high percentage of water saturation through most part of the extensively wide intertidal zone at Gangasagar, the horizontal distribution of meiofauna was also correspondingly great. Further, as recorded in the present study, the concentration of meiofauna at lower levels of flat and sheltered beaches is also known in certain other areas (Smidt 1951; Harris 1972a; Martinez 1975; Dye and Furstenberg 1978; Moore 1979). Animals tolerant to higher temperatures, lower values of water saturation and oxygen tension, appeared to occupy higher tidal levels and this was particularly observed with nematodes, oligochaetes and halacarids. The superficial concentration of meiofauna in fine and poorly drained beaches due to reduced oxygen tensions in the lower layers of the sediment is known in several world situations, particularly in estuarine environments (Perkins 1958; Fenchel and Jansson 1966; Fenchel 1969; Jansson 1969; Coull 1973; Platt 1977; McLachlan 1978; Moore 1979). The concentration of meiofauna in the top 1 cm of the fine sediment in muddy beaches due to lack of oxygen in the lower layers is also well known (Rees 1940; Barnett 1968; McIntyre 1968). Pronounced seasonal changes in the vertical distribution of meiofauna populations due to fluctuations in temperature were observed on the European coasts, where surface concentrations occurred during summer and deep distributions in winter (Renaud-Debyser 1963; Schmidt 1969; Harris 1972b; McIntyre and Murison 1973). As no such seasonal variations in the vertical distribution of meiofauna were observed in the present study, it appears that the seasonal pattern is characteristic only of the temperate areas where freezing temperatures are experienced in the superficial layers of the sediment during winter. The absence of any distinct diurnal vertical migrations of meiofauna in the sand flat might be due to the waterlogged nature of the sediment as reported by Dye (1978).

Little information is available on the seasonal abundance of intertidal meiofauna and peaks are usually noted when observations are made over a full year or more (McIntyre 1969). The observed pattern of two distinct maxima in the present study, a major one in summer and a minor one during winter, is not universal. Correlated with an increase in sea water temperature, one major peak was usually recorded during or immediately after summer, while variations occurred with the other peak (Smidt 1951; Ganapati and Rao 1962; Govindankutty and Nair 1966; Muus 1967; Fenchel 1967; Schmidt 1969; Harris 1972b; McIntyre and Murison 1973; Dye and Furstenberg 1978). However, the meiofaunal densities recorded in the present investigation conform with the earlier studies on Indian coast, which indicated peak numbers of meiofauna during summer, reduced numbers during monsoon and

gradual recovery during winter (Ganapati and Rao 1962; Govindankutty and Nair 1966; Panikkar and Rajan 1970; Damodaran 1974). A similar observation was also made for the population of macrobenthos in a tidal creek on Sagar Island (Bhunja and Choudhury 1981). Lower salinities experienced during monsoon months appear to be unfavourable for the population increase and the seasonal abundance of meiofauna seems to depend mostly on changes in the hydrobiological conditions (figure 5). Low temperature and salinity conditions in the habitat were associated with nematode increase, while their higher values favoured copepods. The hydrographic cycle is probably related with their reproductive activity.

The quantitative data available on meiofauna of the fine intertidal estuarine sediments are presented in table 5, which indicate that the densities of meiofauna populations recorded on Sagar Island are considerably lower than those of the other areas. The present values approach only those of Smidt (1951) from mud wadden on Danish North Sea coast and Dye and Furstenberg (1978) from muddy sand in Swartkops estuary, South Africa. Estuaries are generally considered highly productive systems with greater meiofauna densities than those of non-estuaries (Coull 1973) and thus, the paucity of meiofauna in sediments of this area obviously needs an explanation. The turbid suspension characteristic in waters of this region is inhospitable for animal life. Further, the highly fluctuating hydrographical conditions and the sediment characteristics as fine grain size, presence of silt-clay fraction, poor drainage, high organic matter, low oxygen content, presence of H_2S , etc appear by no means ideal for the meiofauna to flourish in this estuarine environment.

The Hooghly estuarine system is a positive type with varying salinities experienced upstream up to about 296 km from the river mouth (Saha *et al* 1975). It is known that the number of species and individuals of meiofauna steadily decreases from high saline to less saline areas in an estuarine system (Capstick 1959; Gunter 1961; Coull 1973). Further, Sanders *et al* (1965) reported a pronounced reduction in the number of species in a gradient estuary, a reduction that is not evident in a fluctuating estuary. But as already stated, the present study indicated a distinct decrease in the number of meiofauna species and individuals from areas near the mouth towards

Table 5. Maximum densities (nos./10 cm²) of meiofauna recorded in fine intertidal estuarine sediments.

Locality	Nematoda	Copepoda	Ostracoda	Total meiofauna	Reference
Bristol Channel, Britain	10,440	500	790	11,820	Rees 1940
Mud wadden, Denmark	367	389	12	768	Smidt 1951
Whitstable, Britain	5,200	486	742	5,807	Perkins 1958
Blyth estuary, Britain	2,830	—	—	—	Capstick 1959
Salt marsh, Massachusetts	2,130	—	—	—	Weiser and Kanwisher 1961
Salt marsh, Georgia	16,300	—	—	—	Teal and Wieser 1966
Muddy beach, S. India	3,240	490	63	3,815	McIntyre 1968
Sandy beach, S. India	1,150	448	0	1,960	McIntyre 1968
Sandy beach, S. Africa	955	208	—	1,146	Dye and Furstenberg 1978
Muddy sand, S. Africa	329	13	—	345	Dye and Furstenberg 1978
Sandy beach, Sagar Island	218	134	11	342	Present study
Muddy beach, Sagar Island	412	32	21	517	Present study

locations at the upper reaches. Thus, the gross distribution of meiofauna in general appears to be the same in both the fluctuating and gradient estuaries, except that the pattern is more pronounced in the latter. However, as the species composition of meiofauna has not been well worked out in the present investigation, these observations could only be of a tentative nature.

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Bioecology of *Zaniothrips ricini* Bhatti (Panchaetothripinae: Terebrantia: Thysanoptera)—A new thrips pest of castor (*Ricinus communis*: Euphorbiaceae)

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Abstract. Studies on *Zaniothrips ricini* relating to sexual and parthenogenetic reproduction, life cycle, soil pupation, effect of RH and temperature on pupation as well as the three types of migration (i) plant to soil (ii) soil to plant (iii) plant to plant and within plants are discussed. Periodicity of infestation on the castor plant as well as the alternative hosts *Datura stramonium* and *Calotropis gigantea* have also been highlighted. The roles of the mantid predator *Haldwania lilliputana* and the larval parasite *Ceranisus menes* are also indicated.

Keywords. Bioecology; thrips pest; *Ricinus communis*; *Zaniothrips ricini*.

1. Introduction

The Panchaetothripinae comprises essentially of leaf infesting species of considerable economic importance. The available information on biological aspects of this group relates to *Caliothrips fasciatus* (Pergande) (Russel 1912; Bailey 1933), *Heliothrips haemorrhoidalis* (Bouche) (Rivnay 1935; Crawford 1940), *Helionothrips errans*, (Williams), *Hercinothrips bicinctus* (Bagnall) (Morison 1957), and *Caliothrips indicus* (Bagnall) (Saxena 1971). The recent discovery of large populations of *Zaniothrips ricini* Bhatti besides indicating its pest nature, host range and life cycle, has also enabled the study of the impact of abiotic and biotic factors on its incidence on castor as well as on its alternate host plants.

2. Material and methods

For life cycle studies of *Z. ricini*, gravid females were reared on single fresh leaves in separate chimneys. Oviposited leaves were kept fresh by placing them in Knop's solution and the '0' day larvae emerging from the eggs were collected in groups (of 5) and reared, inside separate plastic vials provided with a small piece of fresh leaf. Observations were made regularly on their life cycle. To study the fecundity rate, gravid females were reared on limited portions of a fresh leaf. The old leaves were replaced regularly by fresh ones and the eggs inserted inside the old leaf was counted following the method of Gauddoura *et al* (1967). Both mated and virgin females were reared separately, each with 10 replicates, to study the types of reproduction. To collect the pupae from the soil and also to assess their soil preference for pupation, soil from different depths and of different texture were analysed using Berlese dry funnel technique. The effect of temperature and humidity on pupal duration and

emergence was worked out by keeping the cultures inside a BOD incubator at different temperatures under constant optimum humidity (96% RH) for this species or at different humidities but under optimum temperature (28°C for this species). Also, the nature of infestation and interaction of the predator/parasite populations was investigated through regular surveys in Chingelput, South Arcot, North Arcot and Madras districts of Tamil Nadu. Samples were analysed during dry-count techniques of the Delayed Counting method adopted by Irwin *et al* (1979).

3. Biology

Both sexual and parthenogenetic reproductions are characteristic of *Z. ricini* with mating taking place 2 or 3 days after last pupal moult. Males are promiscuous and twist their abdomen during copulation often inseminating more than one female. The mating lasts from 45 sec to 2 min. Sex ratio is not constant in both parthenogenetic and sexual reproduction and also under laboratory conditions. The female-to-male sex ratio in sexually-reproducing individuals was 4:3 while males alone were produced during parthenogenesis. The fecundity was considerably less in parthenogenetic reproduction, each female producing only 20 to 24 eggs, as compared to sexual reproduction, where a female laid an average of 98 ± 5 eggs during its life span. Eggs are peculiar with an operculum like structure at their tip (figure 1), and are inserted along the margin of the veins.

After an incubation period of about 5-6 days the first instar larva emerged and moulted into the second instar larvae after 2-3 days. The duration of the second instar was 4-5 days, the early second instar larvae carrying the excretory bulb at the apex of their abdomen, a feature characteristic of the Panchaetothripine larvae. The active late second instar larvae turn pale white, lose the excretory bulb, and descend to the soil, where they change into the prepupae. After a day they pupate at a depth of 5 mm, preferring clayey soil to loose soil. This is confirmed by the fact that an average of 19 pupae were extracted from the clay soil in an area of 100 sq cm in contrast to an average of 8.5 pupae from loose soil within the same area. Laboratory experiments also proved that the pre-pupa of *Z. ricini* prefers a place within a depth of 5 mm inside soil. After the pupal stage lasting for 5-6 days, adults emerged and lived for about 25 days under laboratory conditions.

3.1 Effect of temperature and humidity

Soil temperature has a direct influence on the pupal duration and the emergence of

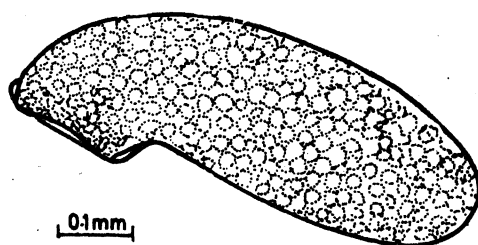


Figure 1. Mature egg of *Zaniothrips ricini*.

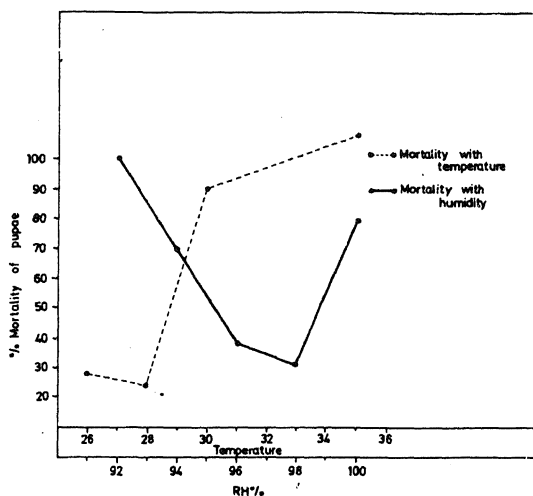


Figure 2. Mortality rate of *Z. ricini* pupae in relation to soil temperature and humidity.

adults which in turn influences the size of the population. The optimum temperature for pupal development was 26-28°C. The pupal mortality rate always increased at temperatures below and above this range, total mortality resulting at temperatures above 35°C. In view of the low mortality rate (35%), 98% RH appeared to be the optimum humidity for *Z. ricini* pupae, as RH of 92% in soil produced 100% mortality and any increase in humidity above this decreased the mortality rate (figure 2). The influence of humidity is indicated by the pupal duration lasting only for five days at RH 94 to 96%, while at 98% it extended by one more day.

3.2 Infestation

As the eggs are inserted adjacent to the veins, the newly emerged first instar larvae aggregate in this region. Infestation of the leaves and feeding by the larvae commence very close to the veins. Subsequently larvae which are now in the second instar stage migrate to the proximal region of the leaf towards the divisions of the palmate leaf. The infested areas appear as whitish patches after 4-5 days. With increase of population, the infestation spreads over almost the whole abaxial side, as a result of which the upper surface of the leaf surrounding the petiolar end dries up causing the leaf to fall off (figure 3 b,c,d).

4. Distribution on castor

Z. ricini are not randomly distributed all over the plant, but restricted to the leaves found between the III to VII recognisable nodes. The first instar and adults are concentrated very close to the veins, while the II instar larvae are mostly scattered throughout. Both adults and larvae are present only on the abaxial sides of the leaves, adults being comparatively more numerous on the fresh leaves (III and IV nodes), than larvae.

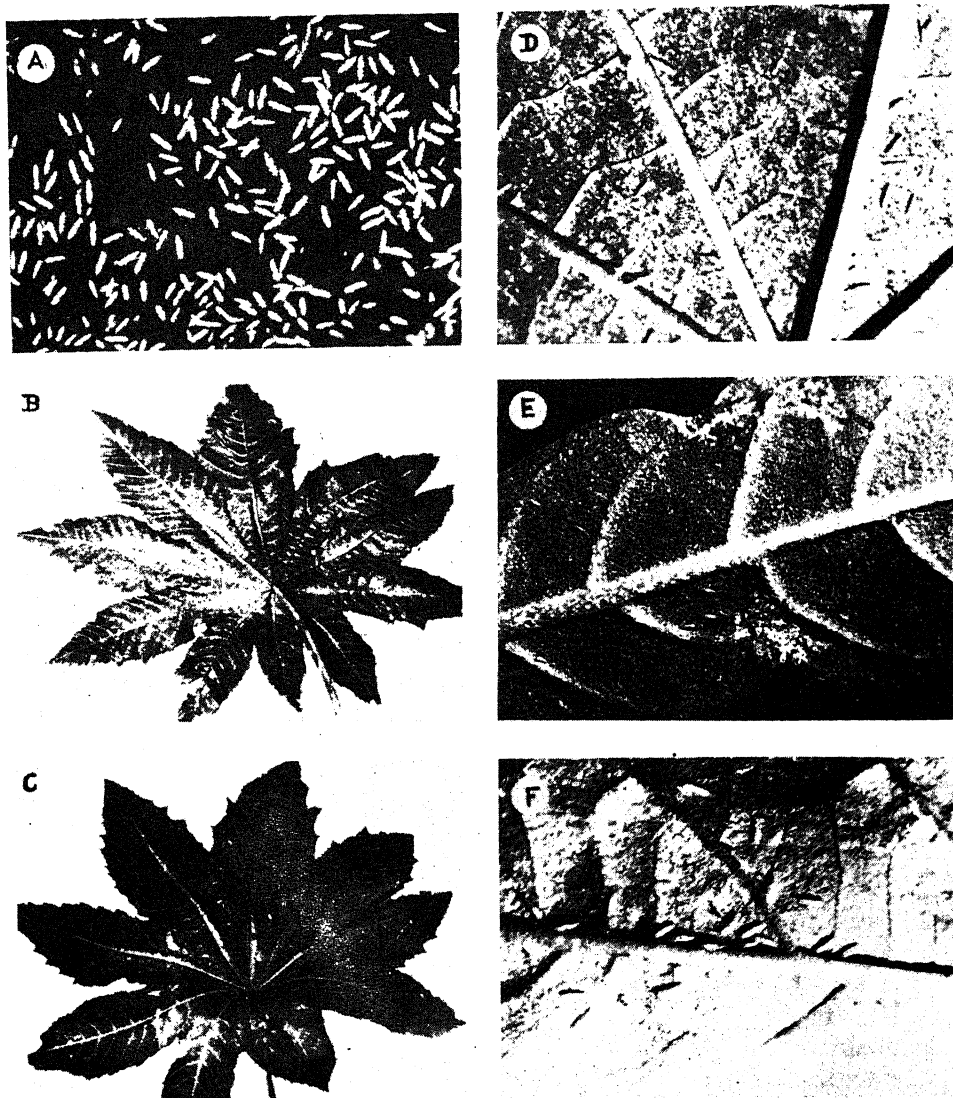


Figure 3. Aerial population and infestation of *Z. ricini*. **A.** Aerial population collected using suction trap **B-D** Infestation on *Ricinus communis* **E** Infestation on *Calotropis gigantea* **F** Infestation on *Datura stramonium*.

4.1 Migration

Three types of migration were exhibited by *Z. ricini*. (i) Migration of freshly emerged adults from the soil towards the plant. (ii) Migration of adults from plant to plant and within plants. (iii) Migration of the IInd instar larvae from the foliage to the soil.

Temperature and humidity appear to have no direct influence on the migration of fresh adults from the soil towards the plant, but light does. The adult emergence from the soil occur during the early hours of the day, the maximum emergence being

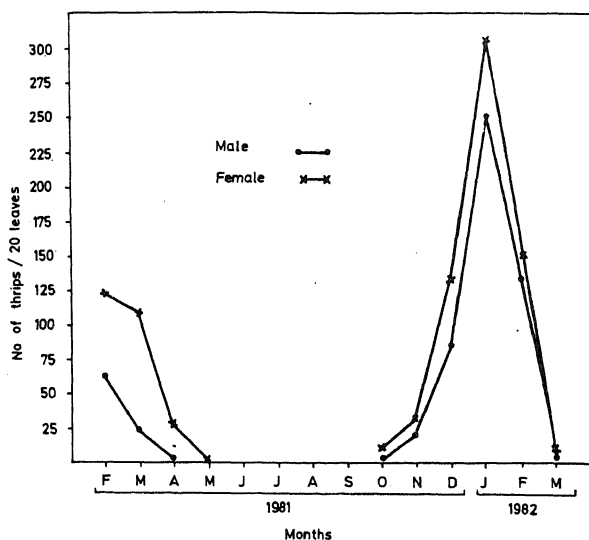


Figure 4. Sex ratio of *Z. ricini*.

between 0080 hr and 1200 hr. Thrips flights were observed when the population reached the maximum, and mass flights during the warmest period of the day (1000 hr to 1200 hr). The active flight period of this species lasted for a few weeks during both the years of study. The late second instar larvae actively moved to the soil as indicated by the presence of considerable numbers in the soil. In the experimental field, the infestation of *Z. ricini* was first noticed in the eastern part of the field. During infestation for about 5 months (November-March) the wind direction was North-East. The thrips infestation also spread in that direction.

5. Natural enemies

Though many predatory beetles, predatory thrips, chrysopids, mantids and nabids were noticed on *Ricinus communis* during different periods of its growing season, *Micraspis cardoni* (Coccinellidae: Coleoptera), *Franklinothrips megalops*, *Mymarothrips garuda* (Aeolothripidae: Thysanoptera), and *Haldwania lilliputana* (Mantidae: Dictyoptera) appeared during the infestation period of *Z. ricini*. The peak populations of these predators synchronised with the decline in the prey population. An average of 20 ± 6 (adults and larvae) thrips were consumed by *Micraspis cardoni* and *Franklinothrips megalops* preyed on 4-5 larvae per day. Nymphal stages of *H. lilliputana* can be considered as the effective predator of *Z. ricini* consuming large number of thrips (96 ± 2) per day and they preferred the active adults to larvae. Though *Mymarothrips garuda* was never found to feed on the larvae or adults, their presence in good numbers along the veins of the leaves suggests that this predatory thrips may feed on the eggs of *Z. ricini*.

The eulophid parasite, *Ceranisus menes* Walker was found to parasitise the II instar larvae of *Z. ricini* where it oviposits a single egg per larva. The parasite completed its life cycle in 8-10 days and then emerged from the host by breaking open the posterior abdominal segments of the pupae.

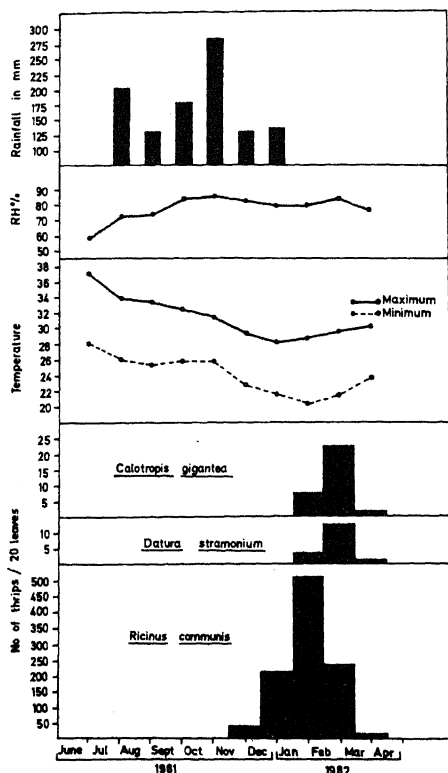


Figure 5. Population trends of *Z. ricini* on the crop and alternate hosts.

6. Alternate host plants

The decline of *Z. ricini* population on castor is immediately followed by the appearance of these thrips on *Calotropis gigantea* and *Datura stramonium*, common weeds found at the sides of castor plantation. In both weed plants the infestation occurs only on the lower surface of the leaves (figure 3 e,f). While only adults were seen on *Datura stramonium*, both larvae and adults appeared to be common on *Calotropis gigantea*. These weeds thus acted as alternative hosts for a short period of one or two months. After March *Z. ricini* is almost completely absent on all the host plants (figure 5).

7. Population trends

In view of its occurrence for 4 to 5 months, *Z. ricini* is considered as the secondary thrips pest species (Ananthakrishnan *et al* 1982) of *R. communis*. *Z. ricini* appeared by October, three months after the plantation of the crop. Till November the population was maintained at a very low level during 1981 and gradually reached its peak by January and declined thereafter, though weekly surveys showed a slight fall during late December. In both the years the population showed a single peak increasing along with temperature during the warmer season. This species was totally absent from April to August coinciding with the off season of the crop from June to July (figure 6).

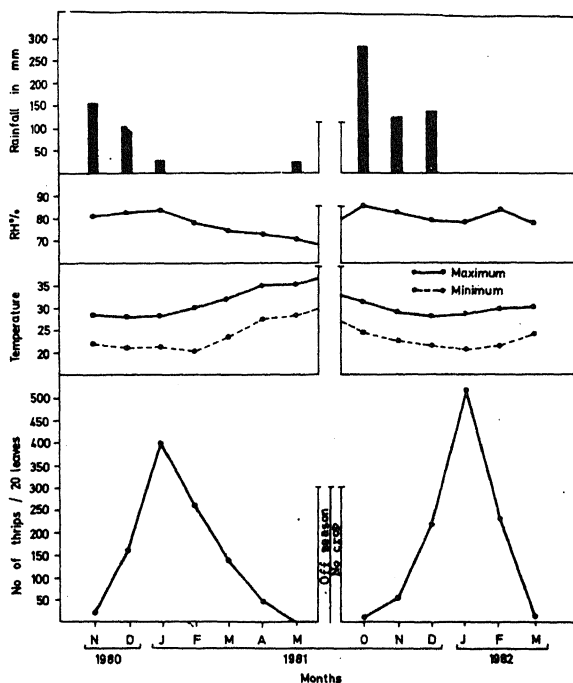


Figure 6. Population trends of *Z. ricini* on *R. communis*.

8. Discussion

The uniqueness of the shape of the eggs of *Z. ricini*, appears contrary to normal pattern. The present study reveals that in 1981, both males and females showed similar trends in the rise and fall of their population. Females are more in number though the trend is similar to that of the males throughout the infestation period. Being capable of reproducing by both sexual and parthenogenetic methods, the existence of more number of females in the natural population (figure 4) indicates that sexual reproduction may be predominant over parthenogenesis in the field. Laboratory experiments indicated that the male to female sex ratio in sexually reproducing individuals is 3:4.

Soil pupation is most common in the terebrantians and normally they move into the soil beneath the host plants (Lewis 1973). In the gum tree thrips *Isoneurothrips australis* Bagnall the larvae fall on the soil from the tree and then move into the soil where pupation takes place (Laughlin 1970), but in the present observation the active late-II instar larvae which lack the excretory bulb descend to the soil for pupation along the stem of the host plant. According to Lewis (1973) the soil dwelling stages of terebrantians are vulnerable to changes in the soil moisture, when the soil becomes too dry or wet. In *Thrips imaginis* Bagnall, when the temperature is 23°C and moisture content is up to 85%, more pupae become adults (Andrewartha 1934). In *Z. ricini*, more pupae become adults at the temperatures between 26-28°C and humidity 94-96%. In both these cases the optimum temperature for maximum emergence was very close to each other, but a total mortality of pupae occurred in the latter species when the moisture content was below 92% and temperatures above 35°C. As such the fall in the number of both males and females at the end of the season may

well be correlated with factors like decrease in humidity and increase in temperature. Like the larch thrips *Taeniothrips laricivorous* Kratochvil (Maksymov 1976) the pupation of *Z. ricini* mainly takes place in the uppermost layer of the soil preferring a place at a depth of 5 mm.

Regarding distributional patterns on leaves, emerging first instar larvae showed a distal primary sub-vascular distribution, whereas the adults exhibited both basal and distal primary sub-vascular distributions. Such distributions are as much in the lines described by Fennah (1963) for *Selenothrips rubrocinctus* (Giard), possibly due to the females laying their eggs close to the veins.

Though this species is positively phototropic, on the host they are strictly restricted to the abaxial side of the leaf where the temperature is comparatively lower than that of the atmosphere and the humidity is higher due to transpiration. This species appears to prefer high humidity (94-96%) and low temperature (26-28°C) so its restriction to the ventral surface could be correlated to these abiotic factors.

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Reproductive strategies and behavioural attributes in some sporophagous Idolothripinae (Tubulifera: Thysanoptera)

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Abstract. An analysis of the behavioural aspects of some sporophagous Idolothripinae in terms of the diversity of reproduction is presented, involving aspects such as oviparity, grades of ovoviviparity and viviparity in *Tiarothrips subramanii*, *Elaphrothrips denticollis*, and *Elaphrothrips procer*. Reproductive diapause in *Loyolaia indica* is also reported.

Keywords. Sporophagous thrips; oviparity; ovoviviparity; viviparity; oocyte; ovariole index; behaviour.

1. Introduction

Contributions to the taxonomy of mycophagous tubuliferous Thysanoptera appear extensive (Ananthakrishnan 1973, 1979; Mound 1971, 1974a,b; Palmer and Mound 1978), but information relating to their biological aspects involving reproductive strategies and behavioural trends appear very restricted, being confined to those of *Bactridothrips brevitubus* (Takahashi) (Haga 1974, 1975, 1980), *Caudothrips buffai* Hood and *Megathrips* sp. (Bournier 1957, 1961), *Diceratothrips* sp. and *Elaphrothrips* sp. (Hood 1940). Ananthakrishnan *et al* (1983) discussed the feeding preferences of some sporophagous Tubulifera in relation to their gut spore composition, mortality rate and post-embryonic development. Although oviparity and ovoviviparity appear common among the Phlaeothripinae (John 1923; Bagnall 1921; Hood 1934, 1938; Hathaway 1938; Haga 1974; Bournier 1957, 1966), facultative viviparity was first reported in *Megathrips lativentris* by John (1923). With ovoviviparity, viviparity and oviparity being evident among the sporophagous idolothripines, an attempt has been made to assess aspects of behavioural and reproductive strategies concerning the following species of sporophagous idolothripines: (i) *Tiarothrips subramanii* (Ramk.) (ii) *Elaphrothrips denticollis* (Bagnall) (iii) *Elaphrothrips procer* (Schmutz) (iv) *Priesneriana kabandha* (Ramk.) (v) *Loyolaia indica* (Anan.).

2. Material and methods

Adults, larvae, pupae and unhatched eggs of the above mentioned species of thrips were obtained from their natural habitat and reared in plastic vials (3.4 × 4.5 cm) and polyethylene bags containing fungus infested host material. Both the fungal spores obtained from the thrips gut as well as from the scraping of the host material were cultured in (i) Potato-dextrose-agar medium (ii) Oat meal-agar medium and (iii) Czepek's medium. The cultured fungi were mounted in lactophenol and stained with cotton

blue (Ananthakrishnan *et al* 1983; Ananthakrishnan and William James 1983). Ovaries from apterous, brachypterous and macropterous individuals, as well as from oviparous, ovoviviparous and viviparous individuals were dissected and oocyte-ovariole indices calculated, using ovariole length/oocyte length. To study the stage of the egg development, longitudinal sections of 8 μ of the lateral oviduct were cut using a rotary microtome.

3. Observations

T. subramanii, *E. denticollis*, *E. procer* and *P. kabandha* tend to be gregarious, the aggregations often comprising oedymorous, normal and gynaeoid males as well as major and minor females in addition to immature stages. The number of individuals in a colony varying considerably depending upon the available fungal food, environmental conditions as well as the incidental type of reproduction. In species such as *T. subramanii*, *E. denticollis* and *E. procer* which do not exhibit alary polymorphism, oviparity, partial ovoviviparity, complete ovoviviparity and viviparity occur, while oviparity alone is evident in such species as *P. kabandha* and *L. indica* which exhibit alary polymorphism.

Both partial and complete ovoviviparity are evident in *T. subramanii*: (i) oocytes developing in the lateral oviduct upto blastoderm formation and subsequent egg laying and hatching occurring within 1 to 2 days (*partial ovoviviparity*). (ii) oocytes developing within the lateral oviduct upto a fairly advanced stage of development as evident by the eyespots and maxillary stylet formation, the eggs hatching within a few hours after laying (*complete ovoviviparity*). When the eggs develop within the lateral oviduct till the fully developed stage as evident by the formation of maxillary stylets, eyespot, legs, etc. the thin chorion surrounding the fully developed larvae ruptures while within the common oviduct and the fully developed larvae are laid—(*viviparity*).

3.1 Oviparity, ovoviviparity and viviparity

Each oviparous ovariole of *T. subramanii* has 4-8 oocytes as compared with 2-9 oocytes in each ovoviviparous and viviparous ovariole. However, only 2-4 oocytes mature at a time on each side in the oviparous ovary, so that an oviparous female tends to lay 4-8 eggs during oviposition. In the ovoviviparous and viviparous ovaries, the lateral oviducts are 1.5 to 12 times longer and 3 to 4 times wider than that of the oviparous forms. The length of the ovariole and that of the basal oocyte within the ovariole are 2 to 2.5 times and 5 times longer respectively in oviparous forms as compared to those of the ovoviviparous and viviparous forms. All the four oocytes on each side of the ovary develop simultaneously in the ovoviviparous ovary, the dimensions of the basal oocytes in the ovariole being 5 times greater in oviparous ovaries.

Gregariousness as well as unfavourable conditions of environment induce ovoviviparity and viviparity in *T. subramanii*, but during favourable conditions or when reared in isolation oviparity occurs. The very short oviduct never retains the eggs in the oviparous ovary. The number of eggs laid by the oviparous individuals is comparatively higher (25-33/female) the incubation period of oviparous eggs lasting 6-7 days. The viviparous individuals produce 7-11 larvae/female.

In *Elaphrothrips denticollis* and *E. procer* as in *T. subramanii* mating occurs soon after emergence. All the grades of ovoviviparity and viviparity are evident in addition to normal oviparity. In viviparous and ovoviviparous individuals of *E. denticollis* and *E. procer* the lateral oviduct is very long nearly 2-5 times longer than that of the ovariole. Fully developed embryos are evident, at the distal end of the lateral oviduct of *E. procer* and in the viviparous forms of this species; the eggs hatched within the common oviduct, the first instar larvae emerging as such while the oviparous females laid 3-10 eggs. The first larvae emerging from the viviparous females appear larger than those from the partial ovoviviparous forms. Fecundity is higher in oviparous *E. procer* with 5-37 eggs/female (1-7 eggs/day) laying in 2-3 days than in the viviparous forms with 5-6 larvae/female (1-3 larvae/day) laying in 2-7 days).

In *Priesneriana kabandha*, where only oviparity occurs, both the lateral oviduct as well as the ovarioles appear very much shorter, but with a very long terminal filament (1253-1485 μ) attached to the salivary glands. The oocyte-ovariole index of the macropterous and brachypterous individuals was 2.4 to 2.6 and 1.5 to 1.6 respectively, and a maximum of eight oocytes are evident at a time in the mature condition. *Loyolaia indica* is a non-aggregating form, occurring singly, with ovaries showing retarded growth in summer months of April-June with no maturing oocytes, indicating its reproductive diapause, while those examined during January-February and September to December revealed their oviparous nature with 4-9 fully developed oocytes. The oocyte-ovariole indices for the oviparous ovary of this thrips species generally ranges between 1-3.7, in most cases being around 3-3.5, whereas the oocyte-ovariole index for the ovoviviparous types of ovaries is highly variable ranging from 5-12 depending upon the degree of ovoviviparity.

3.2 Egg laying sequence and fecundity

The egg laying pattern and the number of eggs laid vary considerably, depending upon the aggregation patterns and sub-social behaviour of the thrips concerned. In *T. subramanii* (30-400 individuals/colony) and *P. kabandha* (40-120 individuals/colony) which form colonies on *Borassus flabellifer* L. and *Eucalyptus globosus* var. *mysoriensis* respectively, lay their eggs in a specific pattern. In oviparous *T. subramanii*, eggs are laid in a linear fashion in the leaf folds or in the laminar regions just near the petioles in order to facilitate the emerging young ones to feed on fungal species such as *Anthostomella consanguinea*, *A. sepelibilis*, *A. phoenicicola*, *Pestalotia algeriensis*, *Alternaria* species and *Melanographium citri* (Ananthakrishnan and William James 1983). The number of eggs laid in each mass varies from 16-42, and the oviposition period ranges from 3 to 5 days, with a fecundity rate of 25-33 eggs/female, the maximum eggs laid per day being 9 and the minimum 3. In *P. kabandha* the eggs are laid in groups of 10-115 in a specific pattern along the underside of the dead barks of *Eucalyptus globosus* (figure 2). The eggs of *E. denticollis* collected from *Areca catechu* and *Tectona grandis* were glued to the surface of the host in a vertical fashion, with the eggs being closer to each other on *T. grandis* than on *A. catechu*, the number of eggs laid being 25-28 and 16-20 respectively. Increased fecundity occurs in the oviparous forms of *E. procer* with 5-37 eggs/female (1-7 eggs/day) than viviparous forms with 5-8 larvae/female (1-3 larvae/day). The oviposition period is higher in ovoviviparous forms (2-7 days) than in the oviparous forms (2-3 days). In *Loyolaia indica* the eggs are laid on the leaf sheaths of *Cynodon dactylon* (Pers) in a

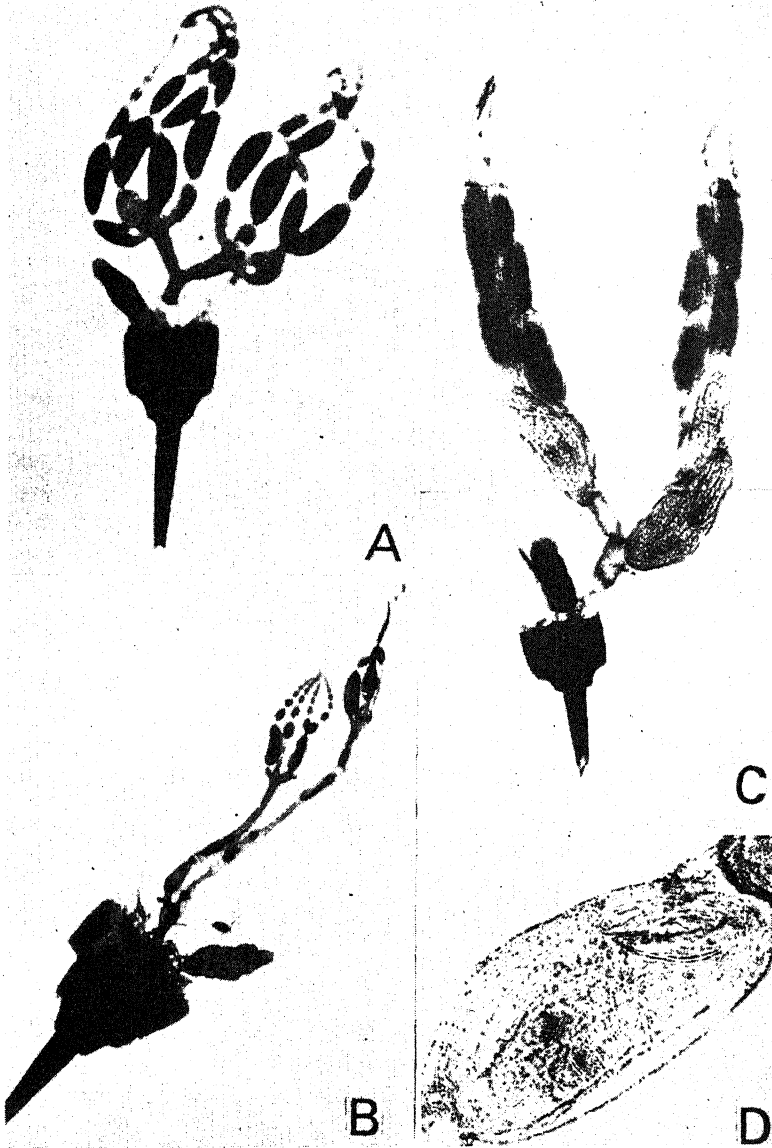


Figure 1. Ovarian types of *Tiarothrips subramanii*. **A.** Oviparous ovary of *T. subramanii*. **B.** Parital ovoviviparous ovary of *T. subramanii*. **C.** Viviparous ovary of *T. subramanii*. **D.** Fully developed larvae in the distal end of the lateral oviduct, showing maxillary stylets, eye spots, etc. (enlarged).

scattered manner, the number of eggs laid by a single female ranging from 4-6 with an oviposition period of 3-4 days.

3.3 Post-embryonic development

T. subramanii, *E. procer*, *E. denticollis* and *L. indica* generally lay their eggs in the leaf folds or in more protected places and rarely on the leaf surface, thus protecting

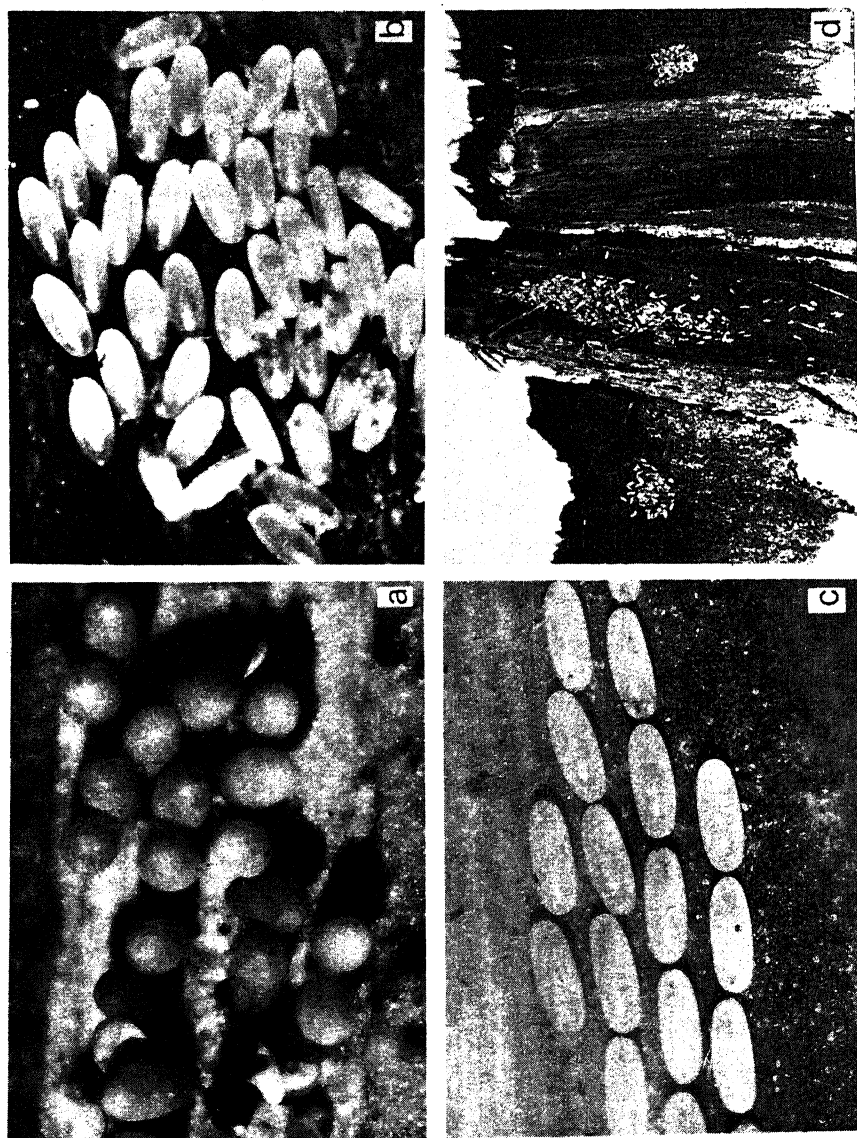


Figure 2. Egg laying pattern of some sporophagous idothripines. a. Egg mass of *Elaphrothrips denticollis*. b. Egg mass of *Priesneriana kabandha* (enlarged). c. Egg mass of *Tiarothrips subramanii*. d. Egg mass of *Priesneriana kabandha*.

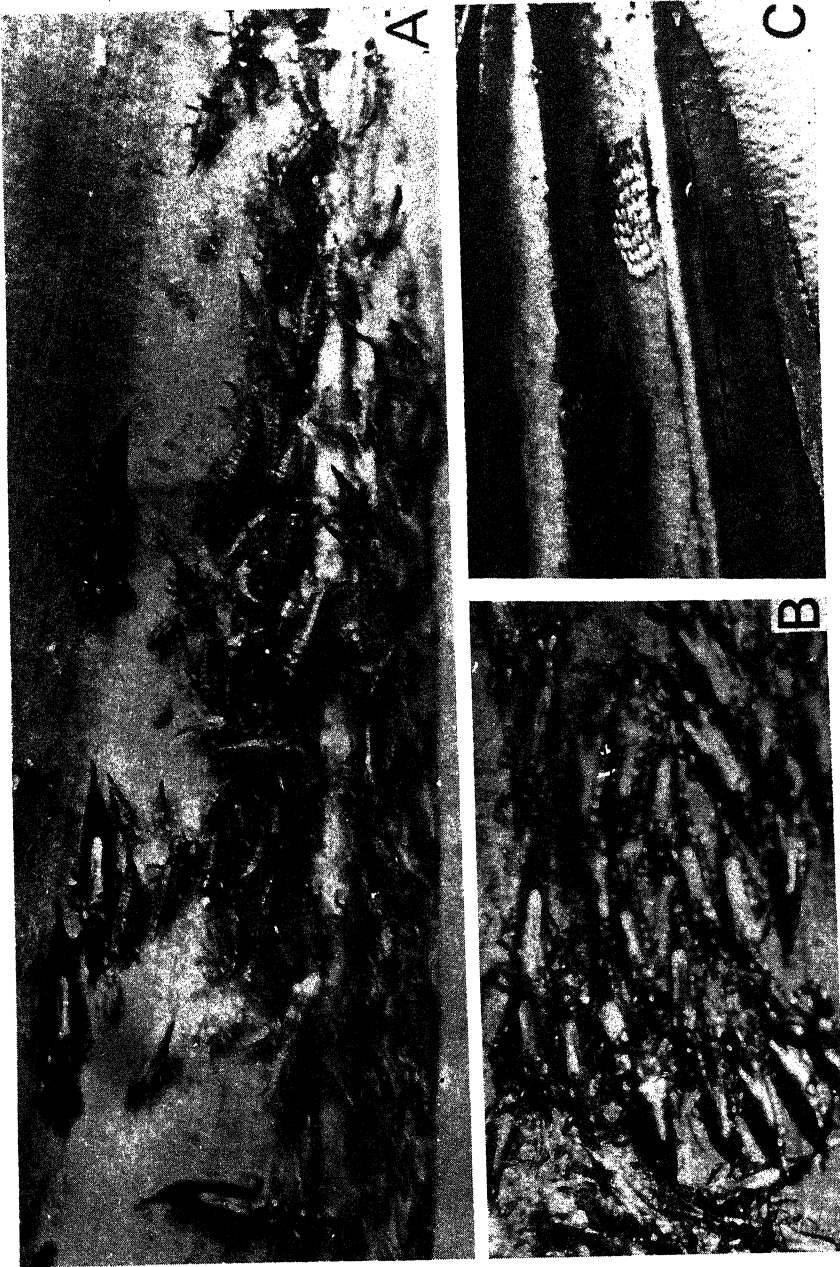


Figure 3. Aggregation and egg protection of some sporophagous idiothripines. A. A colony of *T. subramanii* in dry leaves of *Borassus flabellifer* L. B. Colony of *T. subramanii* (enlarged). C. Oedymorous male of *T. subramanii* guarding the egg mass.

the eggs from direct exposure to sunlight, rain and also from their natural enemies. In these thrips oedymorous males guard the colony as well as the egg mass (figure 3). In *T. subramanii* 4-5 egg masses each with 20-30 eggs occur sometimes within tunnels made by a caterpillar in leaves of *B. flabellifer*. In *P. kabandha* eggs are laid on the underside of dead bark. In colonies of *T. subramanii* and *P. kabandha*, adults, pupae and larvae completely surround the egg mass or stay at the vicinity of the eggs.

The incubation period is determined by the nature of the ovary and the type of reproduction, which in turn are governed by the environmental factors like availability of food, temperature and photoperiod. In partial ovoviviparous *E. procer* incubation period is just one day, whereas in oviparous forms like *L. indica* and *P. kabandha* it is 3-4 days, 6-7 days in *T. subramanii* and 10-12 days in *E. denticollis*, with an average duration of development of 27 and 15.5 days respectively in *E. denticollis* and *L. indica*. The rate of mortality is greater in the first and second larval stages (40-60%) of *E. denticollis* and lesser in first larval stages of *T. subramanii* (4%) (table 1).

3.4 Aggregation behaviour

With the exception of *Loyolaia indica*, other species like *T. subramanii*, *P. kabandha*, *E. denticollis* and *E. procer* exhibit a tendency for aggregation. *T. subramanii* shows linear aggregation within folds of dry leaves of *Borassus flabellifer*, while in other thrips species the aggregation pattern is irregular. The number of individuals in a colony is variable with about 30-165 first larvae, 8-275 second larvae, 9-33 pupae, 20-93 adults and 15-46 eggs in *T. subramanii* and 3-27 first larvae, 27-55 second larvae, 7-19 pupae, 16-27 adults and 10-110 eggs in *P. kabandha*, whereas *E. denticollis* and *E. procer* form comparatively smaller aggregations in *Areca catechu* and dense aggregations on *Tectona grandis*. Aggregations occur throughout the year in *T. subramanii*, *E. procer* and *E. denticollis* which occur only as macropterous forms, whereas *P. kabandha* exhibit close aggregation only while feeding on the fungal species *Cytospora* infesting *Eucalyptus globosus* var. *mysoriensis* and they do not aggregate while feeding on the fungal species *Rhytidhysterium rufula* infesting dry twigs. Aggregation among these thrips is favoured by reduced light and in the fields they aggregate in the dark regions covered by the closed leaflets as in *T. subramanii* and *E. denticollis* and the underside of the dry bark as in *P. kabandha*, with the aggregated condition remaining undisturbed even after continuous rainfall. Both the immature and adults aggregate without any specific orientation and form tiers one above the other with the adults occurring in the topmost layer and the oedymorous males occurring in the margins of a colony. Evidence of territorial behaviour was lacking and the antennectomized males were unable to recognise the colony, did not aggregate and moved away from it.

3.5 Mating behaviour

In a colony, sexually mature males orient towards mature females, the males making a point of contact, initiating a specific response. The point of contact is either from posterior end or at right angles to the thorax of the female or a head to head contact. In a majority of cases the two latter postures result in antennal palpation, whereas in the former condition antennal palpation rarely occurs. Antennal palpation acting as stimulus, enables acceptance by the female and results in the initiation of mating.

Table 1. Post-embryonic development of some sporophagous Idolothripinae

Thrips species	Mean fecundity rate	Mean incubation (in days)	I Larvae			II Larvae			Pre pupa			I Pupa			II Pupa			Mean duration of development
			Mean	Mean dura- tion	% morta- lity	Mean	Mean dura- tion	% morta- lity	Mean	Mean dura- tion	% morta- lity	Mean	Mean dura- tion	% morta- lity	Mean	Mean dura- tion	% morta- lity	
<i>Elaphrothrips denticollis</i>	26.5 (25-28)	11 (10-12)	4.5 (4-5)	50 (40-60)	4 (4)	60 (60)	1 (1)	0 (0)	3 (3)	—	3.5 (3-4)	—	—	—	—	—	27 (25-29)	
<i>Tiarothrips subramanii</i>	29 (25-33)	5.5 (6-7)	2.5 (2-3)	4 (4)	4.5 (4-5)	17 (17)	1 (1)	—	4.5 (4-5)	—	3.5 (3-4)	—	—	—	—	—	16.5 (14-19)	
<i>Priesneriana kabandha</i>	—	3.5 (3-4)	3 (2-4)	22 (22)	3 (3)	35 (35)	1 (1)	—	3 (3)	—	2.5 (2-3)	—	—	—	—	—	16 (14-18)	
<i>Loyolaia indica</i>	5.5 (5-6)	3.5 (3-4)	2.5 (2-3)	—	4.5 (4-5)	18 (18)	1 (1)	—	1.5 (1-2)	—	2.5 (2-3)	—	—	—	—	—	15.5 (13-18)	
<i>Elaphrothrips procer</i>	6.5 (3-10)	1 (1)	2.5 (2-3)	—	10 (8-12)	—	1 (1)	—	1 (1)	—	3.5 (2-5)	—	—	—	—	—	19 (15-23)	

Figures in parentheses indicate range.

Mating success greatly depends to a large extent upon the angle of abdominal flexion serving to engage the genitalia. The oedymorous males which are considerably longer than the females, effectively persuade and mate even after several unsuccessful attempts. The longer abdomen of oedymorous males serves to effectively engage the genitalia in mating, there being more frequent mating among them and the major females. Normal females also effectively bend their abdomen upto 90° along the long axis. Frequent matings were also observed among normal males and normal females, unless disturbed by the oedymorous males. Gynaecoid males were less successful in mating, not being favoured by the females. Whenever disturbed by other thrips the females move away from the males resulting in mating failure. With the initiation of mating it is continued either in an end-to-end posture or side-to-side posture (figure 4)

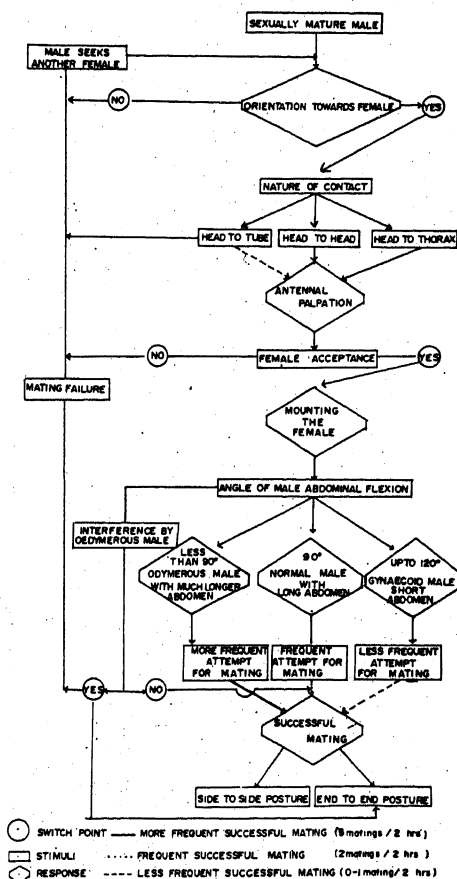


Figure 4. Mating behaviour of *Tiarothrips subramanii*.

4. Discussion

The type of reproduction, whether oviparous or ovoviparous, is determined by factors such as environment, fungal food availability and aggregation behaviour. Oviparity occurs during the more-moist months, when sufficient fungal food is available and a longer incubation period does not result in desiccation. In the drier summer months, reproduction involves the graded types of ovoviviparity and viviparity thus enabling protection of the eggs from dessication and overcoming the fungal food scarcity. *Caudothrips buffai* (Bournier 1957, 1966), which also feed on fungi, exhibit ovoviviparity as an adaptation to shorten the life-cycle, enabling the young ones to complete development before the food disappears. The emergence of live larvae in *T. subramanii*, *E. procer* and *E. denticollis* supports the observations of John (1923) on *Megathrips lariventris* where the eggs usually hatch after 14 days, but occasionally fully formed larvae emerge within few hours of laying and caged females produced larvae without empty shells. Since the ovoviviparous and viviparous eggs are retained in the lateral oviducts for a longer time, the fecundity and oviposition period are considerably short; on the other hand, in oviparous forms the eggs are never retained in the lateral oviduct, so that the oviposition period and fecundity rates are high and as an adaptation the lateral oviducts are long in the different types of ovoviviparous and viviparous forms. As the eggs pass through the lateral oviduct, most of the development occurs as in the partial and complete ovoviviparous forms and with the larvae fully developed as in the viviparous forms. The larger size of the oviparous basal oocyte is attributed to its high yolk content meant for the further egg development subsequent to laying. Hood (1950) and Priesner (1960) showed that in a number of tropical mycophagous species such as *Diceratothrips armatus* Bagnall, *D. princeps* Hood, *Spodothrips amplus* Hood, *Anactinothrips vigilans* Hood, young ones are brought forth from adults, instead of eggs. Bournier (1957, 1966) observed that ovoviviparity is much more common in *Caudothrips buffai*, and noticed fully developed and chitinised larvae from the full-sized eggs dissected out from the gravid females. Viswanathan and Ananthakrishnan (1973b) also refer to the prolonged time spent by the oocyte within the oviduct, the presence of large receptaculum seminis with numerous sperm bundles to avoid the necessity of frequent mating, unusual longevity of females and the ability of eggs to survive desiccation for long time in *T. subramanii* as an adaptation for survival in the extreme conditions.

Loyolaia indica exhibits oviparity throughout the year except during summer (March-August), when they do not reproduce and maturation of oocytes does not occur. Except adults, eggs and immature stages were not noticed in the field, indicating an ovarian diapause condition. Photoperiod, temperature and food availability appear to be the main factors for ovarian diapause in thrips, wherein gut spore analysis of *L. indica* during this diapause stage revealed the gut to be completely filled with spores of *Lojkania cynodontifolii* (Ascomycete). The occurrence of oviparity during the rest of the year could be attributed to its non-aggregation behaviour, as well as their ability to migrate to alternative hosts. *L. indica* which occurs only in dry fungus infested clumps of *Cynodon dactylon* during the favourable periods, also occurs on other similar dry fungal-infested alternative hosts such as *Chloris barbata*, *Panicum repens* as well as dry twigs during late summer and early wet seasons (July-October) where they feed on the microconidia of *Fusarium oxysporum* and spores of *Penicillium* sp. This change of host during unfavourable conditions is perhaps due to the scarcity

for fungal spores of *Lojkania cynodontifolii* in their original hosts. On the whole non-aggregation behaviour, migration to alternative hosts and availability of fungal food throughout the year appear responsible for its oviparous type of reproduction throughout the year.

Oviparity occurs throughout the year in *Priesneriana kabandha* which forms close aggregations and exhibits a sub-social life on the undersurface of the dead barks of *Eucalyptus globosus* var. *mysoriensis* throughout the year except in summer where they migrate to nearby thorny dry twigs, where they do not form colonies. Thus change in aggregation behaviour, habitats and food fungi appear to be responsible for the density of the colony and surplus of fungi for feeding and rendering oviparity possible even in summer. All polymorphs occur in a colony throughout the year except during the end of the summer and early rainy season when only macropterous forms are evident. Here the production of macropterous forms appear to be due to the lack of sufficient fungal spores of *Cytospora* sp. (Coelomycete), resulting in migration of this thrips to other dry twigs infested with *Rhytidhysterium rufula* (Coelomycetes). Hood (1940) observed that *Hoplothrips flumenelles* exhibiting intraspecific ecological succession, the macropterous forms succeed the apterous forms. He also showed that in a colony nearly every individual is brachyperous and observed that "the immature organism carries within itself an environmentally-activated mechanism which assures a means of escape from the parental home, should inevitable changes make it no longer suitable to the life of the organism or itself". Earlier studies in bark dwelling phlaeothripids such as *Hoplothrips* sp., *Hoplothrips flumenellus* by Hood (1940) and *Megathrips* sp., and *Cephalothrips* sp. by Bournier (1961) also showed that brachypterae are produced when the fungi are fresh and vigorously growing, while macropterae are produced as they age and deteriorate.

In sub-social species such as *T. subramanii*, *E. procer*, *E. denticollis* and *P. kabandha*, females in a colony lay their eggs in a sequence. Each female of *T. subramanii* lays its eggs in a separate linear sequence of three, where as in *P. kabandha* colony all the females lay in a single mass. Both *T. subramanii* and *P. kabandha* when they form aggregations, the oedymorous males guard and protect the colony and egg mass, thus tend to exhibit division of labour. Bagnall (1915) also observed that *Elaphrothrips brevicornis*, a West Indian species, sits over her eggs after laying them on the leaves and protects the eggs from the predators.

The duration of the post-embryonic development varies with the thrips concerned as well as with the fungi they feed on. The highest mortality rate during the second larval stage may be due to longer duration and higher food requirements for further development. Earlier observations (Ananthakrishnan *et al* 1982) showed that the duration of the post-embryonic development and mortality rate vary with the host fungi.

An extreme degree of aggregation and sexual selection was noticed among the colony forming sporophagous idolothropines. Though the variation in numbers among the different polymorphs varies with season, there seems to be a balanced proportion of the extreme morphs (Ananthakrishnan 1964). Ananthakrishnan (1973) also showed the possibility of a genetic factor being involved in the production of these morphs. The nature of aggregation and the population in a colony greatly depend on the fungal food availability which in turn depend upon the environment. In all these species the antennae are found to play an important role in recognition of thrips and its colony. The females tend to exhibit a preference towards the oedymorous males

with enlarged forefemora, very long third antennal segment and long cephalic process, thus exhibiting sexual selection. Hence the success of reproduction to a very large extent appears to be due to sexual selection operating in the colony. This supports the observations by Mayr (1972) and Johnson (1982) where secondary sexual characteristics such as exaggerated size of the individuals or the parts of the body or colouration tend to occur in males presumably as a result of sexual selection, reflecting the competition of males among the female choices. Ananthkrishnan (1972) reported that the mycophagous thrips provide very good instances for studies of sexual selection in view of its large size and occurrence of sex-limited polymorphism. The large size in males appears to confer a greater probability of success in thrips reproductive investments and the behaviour of the female to choose a large mate will be favoured and maintained (Johnson 1982).

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Cytochemical changes in the silk gland cells of *Pericallia ricini* (Lepidoptera: Arctiidae)

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Abstract. Cytochemical changes involving PAS—positive and metachromatic substances have been studied in the silk gland cells of the moth *Pericallia ricini* during growth and atrophy. The distinction between the silk-periphery and silk-core on the basis of metachromatic staining has been traced even in the second instar stage for the first time. PAS—reaction differentiates the same in the prepupal stage. The secretion of two silk constituents goes on simultaneously in the gland cells. The conjugation of PAS—positive polysaccharide group to silk protein takes place in the apical cytoplasm of the gland cells. The core silk secretion predominates in the prepupal stage. Reduction in the amount of carbohydrate material in the basal cytoplasm of the silk gland cells from first instar to prepupal stage occurs with their simultaneous concentration in the apical region of the cells. The degree of metachromasia increases progressively in advancing instars.

Keywords. PAS-positive and metachromatic substances; silk secretion; growth; atrophy; silk periphery; silk core.

1. Introduction

The presence of PAS - positive and acidic mucosubstances in the silk gland cells of *Calpodex ethlius* has been observed (Wiley and Lai-Fook 1974). These were confined to the basal cytoplasm and were characterised to be responsible for cohesiveness and viscosity of the silk contents in the lumen of the gland. Many workers (Voigt 1965a; Akai and Kobayashi 1966; Beams and Sekhon 1966; Tashiro *et al* 1968; Akai 1971; Engster 1976) suggested that carbohydrate moieties are added to the protein molecules of silk in later packaging prior to their extrusion into the lumen. They, however, failed to correlate basal localization of carbohydrate substances with their binding to the protein molecules in the apical cytoplasm.

Since the cytochemical picture of silk gland cells is conflicting and vague, it was desirable to study the localization and role of carbohydrate substances in all the developmental stages of the Indian moth, *Pericallia ricini* in presecretory, secretory and post-secretory phases of the silk gland cells.

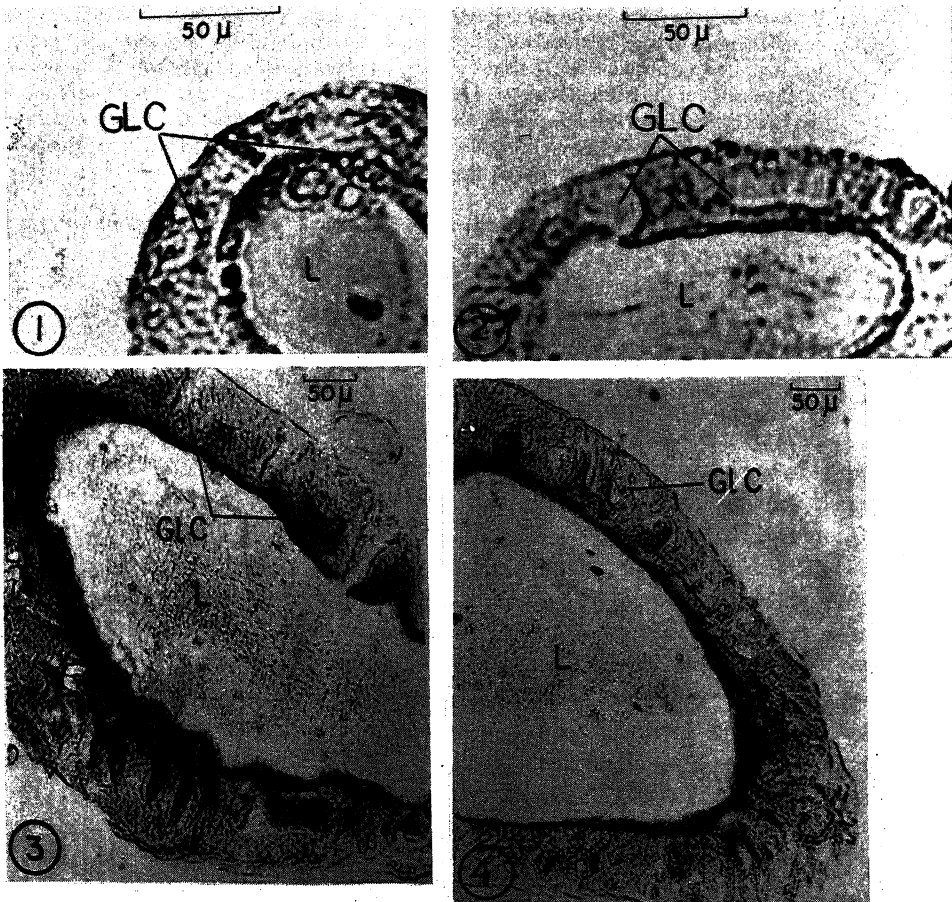
2. Material and methods

The larvae of *P. ricini* were collected from fields around Allahabad and reared in the laboratory. The silk glands of different instar larvae and early pupae were excised, cut into small pieces and fixed in 10% formalin for 24 hr. Part of this material was embedded in gelatin and frozen, 10 µm thick sections were cut out with the freezing

microtome. The other part was dehydrated in graded alcohols, embedded in paraffin and sectioned at 8 μm . The periodic acid-Schiff (PAS) method (McManus 1946) as described by Pearse (1968) was employed to detect the PAS-positive material. For metachromatic substances toluidine blue (Spicer 1960) and Schmorl's thionin methods (Pearse 1968) were employed. Control sections were processed by acetylation (Lillie 1954a).

3. Results

The cells of the silk gland of *P. ricini* are almost similar with respect to their size, nucleo-cytoplasmic ratio and shape of the nuclei. PAS-positive material in the gland cells of the second instar larva is greater than the first instar and is in greater concentration at the apical (facing the lumen) and basal (facing the basement membrane) ends of the cells (figure 1). In the middle region of the gland cells, the cytoplasm contains sparse concentration of polysaccharides.

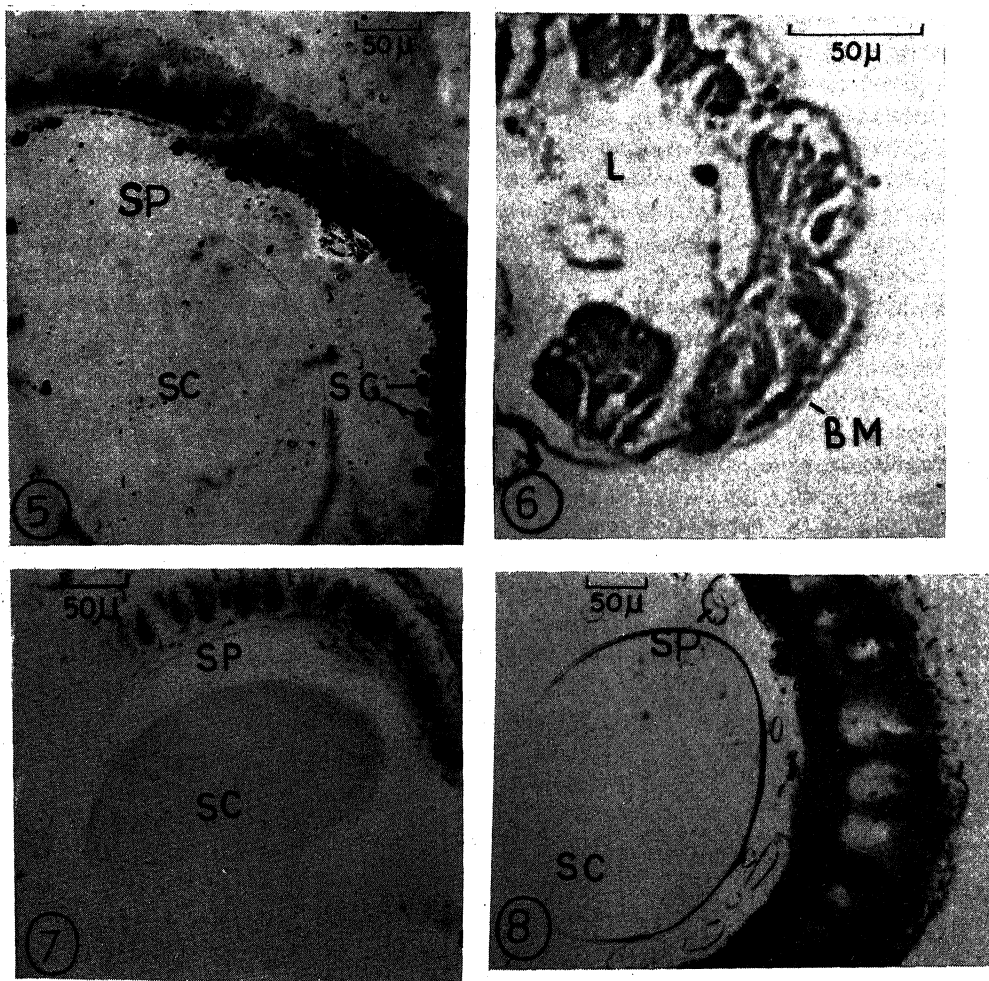


Figures 1-4. Silk gland cells; 1. Second instar larva showing PAS-positive substances confined mainly to the apical and basal regions. 2. Third instar larva showing PAS-positive substances. 3. Fourth instar larva showing PAS-positive substances. 4. Fifth instar larva showing greater PAS-positivity in the apical cytoplasm. L, lumen; GLC, gland cells.

Cells of the spinning gland of the third instar larva (figure 2) show less periodate consumption in the middle and basal cytoplasm. The apical end shows preponderance of reactive glycol groups.

In the following stage, as seen in figure 3, the cytoplasm around the nuclei becomes almost free of carbohydrate material. In the basal region of the gland cells a weak reaction indicates reduced amount of PAS- positive substances. While carbohydrate substances decline in other parts of the cells, they concentrate more in the apical region. Gland cells of the fifth instar larva depart little from those of the previous stage in their cytochemical details except that the reduction of PAS- positive substances in the basal region and their concentration in the apical region continues progressively (figure 4).

In the prepupal stage, substances showing PAS- positiveness disappear from the peripheral region of the silk gland cells (figure 5). The intensity of the stain developed



Figures 5-8. Silk gland cells. 5. Pre-pupa showing weak PAS-positive stain. 6. During pupal period. Weak PAS-positivity in the degenerating cells. 7. Third instar larva showing weak metachromasia. 8. Fifth instar larva showing increased metachromatic reaction. BM, basement membrane; SC, silk core; SP, silk periphery; L, lumen.

in the apical region is also reduced. It is in this stage that the secretion of silk in the cells is maximum.

A weak PAS-positive stain develops in the cytoplasm of the gland cells during the pupal period (figure 6). With the signs of glandular degeneration appearing in the cells substances having PAS-positiveness get further reduced. In advanced stages of degeneration a very faint stain in the cells indicates presence of exceedingly few carbohydrate groups amenable to periodic acid oxidation. The basement membrane is PAS-negative at all stages of development.

The distinction of silk core from silk periphery becomes apparent in the late fifth instar stage. Before that the two zones of silk are undefinable. A weak PAS-positive stain develops in the silk in the second, third and early fourth instar stages. Later in the fifth instar and in the succeeding stage when the silk zones become distinguishable, it is the silk periphery alone which gives faint PAS-stain, the silk core, which forms the bulk of the silk, is entirely PAS-negative. Control sections of the silk glands of different stages were treated with a mixture of acetic anhydride and pyridine prior to staining. A negative PAS-reaction was observed in all the sites of the silk gland cells (during larval as well as pupal stages) which were otherwise positive in normal untreated sections.

The cells of the silk gland of second and third instar larvae show weak metachromasia around the nuclei indicating presence of a very small amount of anionic groups in this region (figure 7). In the rest of the cytoplasm no appreciable toluidine blue metachromasia develops. The silk periphery also shows presence of metachromasia-exhibiting carbohydrates. The central part of the silk comprising the core, does not yield metachromatic reaction. It is noteworthy that the distinction between the two zones of silk, periphery and core, in the lumen of the gland is recognisable on the basis of metachromasia in these early stages of development. PAS-staining failed to identify these two zones even in the fourth and fifth instar stages.

In the following instars, the gland cells exhibit greater degree of metachromasia around the nuclei as well as in the basal and apical cytoplasm (figure 8). The position remains almost the same in the cells during the fifth instar stage. Corresponding increase in the degree of metachromasia is noticeable in the silk periphery also in these stages of larval development.

In prepupal period pink colour, developed as a result of metachromatic reaction (both in the perinuclear cytoplasmic zone and the basal and apical cytoplasm) is maximum (*cf.* previous stages). Therefore, the localization of metachromasia-exhibiting substances in the cells of the silk gland of *P. ricini* remains almost unaltered throughout the larval development. A slow increase in the same region of the cells, is, nevertheless, observed from the second instar onwards to prepupal-stage. The basement membrane did not yield metachromatic reaction.

In pupal period the substances yielding metachromatic reaction do not remain confined to the perinuclear cytoplasm in the degenerating silk gland cells. They get dispersed randomly in small groups and give faint pink colour reaction. These substances later disappear from the cytoplasm of the gland cells.

4. Discussion

Bradfield (1951) had reported that the larval silk gland cells of *Bombyx mori* and *Cossus cossus* did not possess appreciable amounts of glycogen and other poly-

saccharides. However, he noticed differences between the staining properties of silk in the anterior and posterior parts of the caterpillar's silk glands, although he could not explain the reason for the staining differences. According to Mercer (1954) and Voigt (1965a, b) there is distinction between the two silk components in the silk glands of *B. mori* and these two components are secreted by different cell types having different types of secretory granules. Wiley and Lai-Fook (1974) investigated the histochemistry of the silk glands of the fifth instar larva of another lepidopteran *Calpodex ethlius* and showed that the cells of the silk glands show PAS-positive and acidic mucosubstances localized in large patches in the basal regions of the cells. They further observed varying cohesiveness and viscosity of the contents (silk) in the lumen of the gland. Present investigations on the silk glands of *P. ricini* reveal that PAS-positive substances are present in appreciable amounts even in the second instar stage. Moreover, there is remarkable variation in the pattern of distribution of these substances in the cytoplasm of the gland cells. Initially carbohydrate-containing substances are confined to both the basal and apical regions of the cells, later the apical region becomes predominantly rich in comparison to the basal region, where reactive polysaccharide groups get progressively decreased. In prepupal stage, when the secretion of silk in the cells is at its zenith, the peripheral region of the cells is devoid of PAS-positiveness, and it is only the luminal (apical) region of the cells where PAS-positive substances are exclusively concentrated, although the stain is less intense than that in the preceding early fifth instar stage. All the stages of development (from the first instar to early pupa) of *P. ricini* were studied to determine whether there is any distinction between the silk zones in the lumen of the gland, and if so, at what stage the distinction becomes apparent. It was found that it is in the prepupal stage when the two components of the silk secretion become distinguishable, and that it is the silk periphery alone which yields a faint PAS-positive reaction. The silk core forming the bulk of the silk is entirely PAS-negative. With toluidine blue staining, however, metachromatic reaction distinguished clearly the two silk components in the lumen of the silk gland even in the second instar stage. It indicates that metachromatic carbohydrate substances are being secreted and conjugated with proteins of the silk, and aggregate in the lumen of the gland in contact with the cellular lining right from the initial stages of silk secretion. Also the metachromasia-exhibiting carbohydrates appear in larger amount in the silk periphery in prepupal stage than in the preceding stage (fifth instar) of development.

The reduction in the amount of carbohydrate contents in the basal region of the gland cells and their concentration in the apical one points *inter se* to their transportation from the former to the latter region of the cells. Since this process continues progressively with increased activity of the cells in relation to silk secretion, the PAS-positive substances are incorporated into the secretion of the cell. It is believed that the polysaccharide moieties get conjugated to silk proteins prior to their escape from the cell. This phenomenon probably takes place in the apical region of the cell. This is evident from the fact that some silk globules, escaping into the lumen of the gland, show weak PAS-positiveness indicating that carbohydrate group has been attached to the main protein group. It is, thus, found that in *P. ricini* (i) the two silk constituents (silk periphery and core silk) are secreted simultaneously in the gland cells, (ii) the PAS-positive polysaccharide group conjugates with silk protein in the apical cytoplasm (iii) the core-silk secretion (consisting of PAS-negative groups) predominates in the prepupal stage.

These findings are in agreement with those of Voigt (1965a), Akai and Kobayashi (1966), Beams and Sekhon (1966), Mac-Gregor and Mackie (1967), Tashiro *et al* (1968), Akai (1971) and Engster (1976), in that the secretory mechanisms in the silk producing cells of lepidopterans and trichopterans have much in common and that carbohydrate moieties to the protein molecules are added in later packaging before their extrusion into the lumen.

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Taxonomic value of torus longitudinalis and valvula cerebelli in four species of *Amblypharyngodon* Bleeker (Pisces, Teleostei, Cyprinidae)

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Abstract. The configuration of valvula cerebelli and torus longitudinalis shows distinct variations at the level where the tractus mesencephalocerebellaris posterior establishes full connection with the granular valvula in the four species of *Amblypharyngodon*. The taxonomic value of the configurational variations of mesencephalon of the four species has been discussed.

Keywords. *Amblypharyngodon chakaiensis*; torus longitudinalis; tractus mesencephalocerebellaris; valvula cerebelli; taxonomic value.

1. Introduction

Various taxonomists have attempted to use brain morphology as a taxonomic character to identify phylogenetic affinities (Lissner 1923; Svetovidov 1953; Uchihashi 1953; Uchihashi and Tsuge 1953; Yamaguchi 1961; Miller and Evans 1965). Lissner (1923) proposed a scheme of classification using the brain patterns and finally concluded that ecological factors have such a profound influence that brain lobes could not be used in classification. According to Kuhlénbeck (1975) the configuration of the ganoid and teleostean mesencephalon displays more (taxonomically-related) variations than that of elasmobranchs. In teleosts particularly, wide differences in external morphology and internal structural arrangements are manifested. Recently Tandon and others (Tandon 1978, 1980; Tandon and Kaur 1979) showed that the configuration of the tori longitudinales and valvula cerebelli at the level where the tractus mesencephalo-cerebellaris posterior establishes a full connection with the granular valvula is of some taxonomic importance in the light of the studies on *Barilius* and *Channa*. According to Ariens Kappers *et al* (1936) this tract arises in the nucleus lateralis valvulae. In this paper the utility of these observations has been assessed in species of *Amblypharyngodon*.

2. Material and methods

Live fish were brought to the laboratory and anaesthetised. The entire brain was dissected and fixed in aqueous Bouin's fluid. Paraffin sections (6-8 μ thickness) were cut and stained with Heidenhain's iron haematoxylin and counterstained with alcoholic eosin. Ten specimens (5 male and 5 female) of each of the four species were studied.

The specimens measured 85 ± 1 mm in total length and were of comparable state of maturity viz. maturing stage.

3. Results

The tori longitudinales arise from the central grey zone of the optic tectum, the two tecta are connected by an intertectal commissure which passes over the tori and some fibres of the commissure may terminate in the mass of the tori. The tori hang in the optocoel. The configuration of the tori and valvula cerebelli at the level where the tractus mesencephalo-cerebellaris posterior establishes full connection with the granular valvula varies in the species of *Amblypharyngodon* and the details of the observations are presented below.

3.1 *Amblypharyngodon mola* (Ham) (figure 1)

The tori are small, triangular in shape and are widely separated from each other. The base of the tori is separated from the periventricular layer by the intertectal commissure. The optocoel between the tori and valvula cerebelli is quite wide.

The valvula consists of granular and molecular valvula. The granular valvula covers molecular valvula laterally and ventrally. On the ventral side the granular valvula is discontinuous at the point where there is a furrow extending to the centre of the central molecular valvula. The central molecular valvula is circular and the lateral molecular valvulae, resembling the distal segment of the human thumb, projects inwards leaving no vacant space between them.

3.2 *Amblypharyngodon microlepis* (Bleeker) (figure 2)

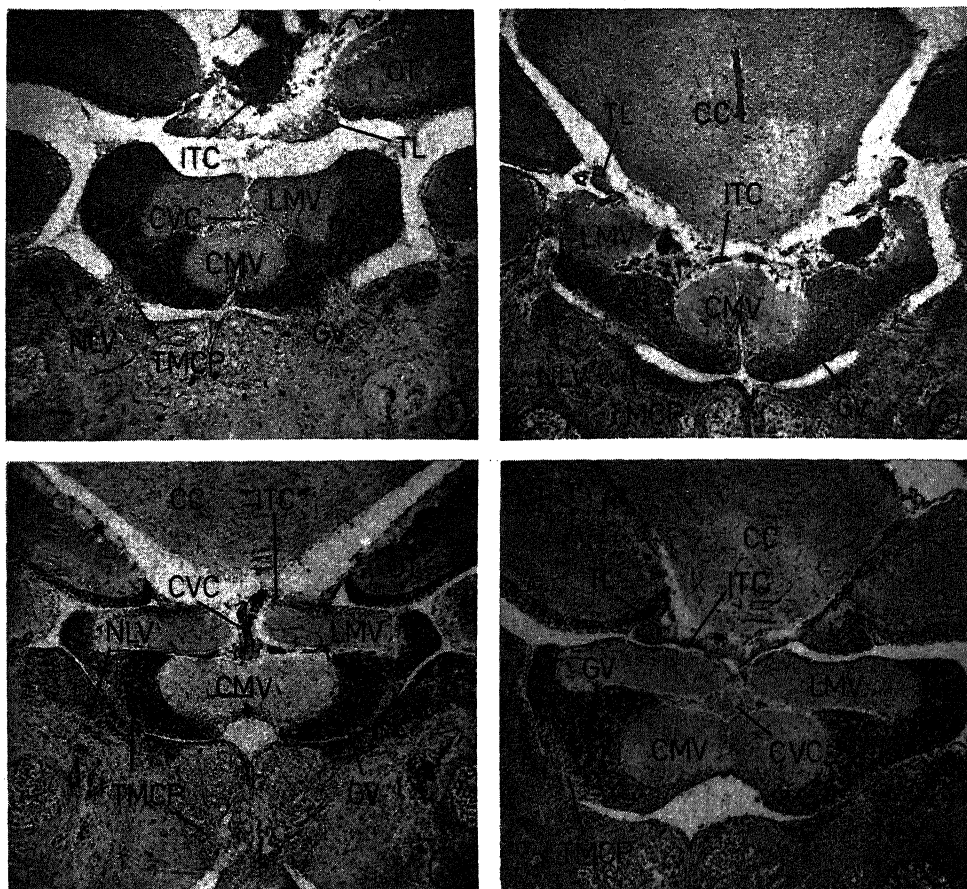
The tori appear like patches of granular matter and are separated very widely from each other and the periventricular layer by a thin intertectal commissure. The optocoel between the tori and valvula cerebelli is narrow and limited to the ventrolateral side and the intertectal commissure is closely placed on the valvula leaving no part of the optocoel between them.

The valvula is better developed in this species than in *A. mola* and is 'V' shaped. The granular valvula is dominant and covers the molecular valvula on the ventrolateral sides. The inward growth of the granular valvula is very prominent. The central molecular valvula is approximately double the size of the lateral molecular valvula which has no inwardly projecting process. Consequently the dorsal boundary of the *Cavum cranii* is placed further up and is formed of a thin intertectal commissure. The lateral molecular valvula is triangular in shape with rounded corners.

3.3 *Amblypharyngodon melettinus* (Cuv. and Val.) (figure 3)

The tori do not extend up to the level where tractus mesencephalo-cerebellaris posterior establishes full connection with the granular valvula. This is a unique feature of *A. melettinus*. The intertectal commissure is closely placed on the valvula leaving no space between them.

The molecular valvula is differentiated into a central bilobed molecular valvula and thick rectangular lateral molecular valvula and between them lies the *Cavum*



Figures 1-4. Transverse section passing through the region where the tractus mesencephalo-cerebellaris posterior establishes full connection with the granular area of the valvula cerebelli in the four species of *Amblypharyngodon* $\times 300$. 1. *A. mola* (Hamilton). 2. *A. microlepis* (Bleeker). 3. *A. melettinus* (Cuv. and Val.). 4. *A. chakaiensis* (Babu and Nair).

Abbreviations

CC—Corpus cerebelli; CMV—Central molecular valvula; CVC—Cavum cranii, GV—Granular valvula; ITC—Inter tectal commissure; LMV—Lateral molecular valvula; NLV—Nucleus lateralis valvulae; OT—Optic tectum; TL—Torus longitudinalis; TMCP—Tractus mesencephalo-cerebellaris posterior

cranii. The granular valvula as usual covers the molecular valvula on the ventrolateral sides leaving a gap at the mid-ventral position and in addition by a thin layer on the dorsal side of the valvula.

3.4 *Amblypharyngodon chakaiensis* Babu and Nair (figure 4)

The tori are small triangular pieces widely separated having a very narrow cavity of the optocoel between them and the valvula cerebelli. The tori are separated from the periventricular layer by a prominent intertectal commissure. The intertectal commissure

has a downward projecting conical process pointing towards the median gap between the lateral molecular valvulae.

The valvula leaves a conspicuous space of the optocoel ventrally. The granular valvula has a very prominent inward growth which separates the central molecular valvula from the lateral molecular valvula. The *Cavum cranii* is well marked. The central molecular valvula is a bilobed structure with the lateral molecular valvulae rod-shaped.

4. Discussion

Svetovidov (1953) supported the idea that brain patterns could be used as diagnostic characters in a number of systematic groups. He also believed that in comparative studies among species from distantly related groups ecological influences on the brain lobes are 'over-shadowed' by differences caused by peculiarities of the brain. Miller and Evans (1965) suggested that extreme caution must be exercised in using brain pattern alone to indicate phylogenetic affinities. They showed that brain patterns in *Thoburnia rathoea* and *Pantosteus delphinus* are similar because of parallelism in habit preference and feeding behaviour although they belong to different types of *Catastomidae*. Uchihashi (1953) and Uchihashi and Tsuge (1953) related that in teleostei ecological differences strongly influence the external shape of the brain. The present study on *Amblypharyngodon* reveals that there are conspicuous variations in the configurations of the torus longitudinalis and valvula cerebelli even in closely-related species exhibiting parallelism in habitat preference and feeding behaviour. In this case ecological influences are not seen to obscure the diversity caused by peculiarities of the brain. According to Miller and Evans (1965) comparison of brain patterns appears to be most useful taxonomically at the subfamily or tribe level, though they are instructive in numerous cases at the generic and specific levels. But Svetovidov (1953) identified only distantly-related groups based on the peculiarities of the brain. More information is required to raise the status of the configuration of the torus longitudinalis and valvula cerebelli at the level where the tractus mesencephalo-cerebellaris posterior establishes full connection with the granular valvula, as a taxonomic criterion to identify phylogenetic affinities. Till then we share the views of Tandon (1978) that the configuration of the above structures can be taken as additional parameters in establishing taxonomic relations.

A close scrutiny of the results of the present study suggests that of the four species studied, *A. mola* is the 'ancient species'. The comparatively poor development of the valvula cerebelli and the nature of the central and lateral molecular valvulae point to this conclusion. The similarity in the configuration of mesencephalic structures in *A. melettinus* and *A. chakaiensis* shows that these two species are closely related. Babu (1981) in his studies on the morphological peculiarities of the four species also arrived at the same conclusion. But *A. microlepis* exhibits very conspicuous configurational variations from the other three species. The nature of the lateral molecular valvula and its association with intertectal membrane are characteristic. On examining the morphological characters Babu (1981) observed *A. microlepis* to be the more specialized species.

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Histochemical distribution of tetrazolium reductases, dehydrogenases and lipids in the follicular wall of normal and atretic follicles in the ovary of the Indian gerbil *Tatera indica* (Muridae: Rodentia)

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Abstract. The comparison of the histochemical features of the follicular wall in normal and atretic follicles of corresponding stages in the ovaries of Indian gerbil *Tatera indica* revealed conspicuous changes in amount of lipids and activities of NADH- and NADPH tetrazolium reductases, Δ^5 - 3β -hydroxysteroid dehydrogenase and 17α -hydroxysteroid dehydrogenase and succinate- and lactate dehydrogenases in the granulosa layers during follicular growth and atresia. The thecal layers showed a progressive increase in their amounts during follicular growth and these enzymes are not affected significantly by atresia. Atresia resulted in increased amounts of lipids in the granulosa cells and decreased activities of succinate- and lactate dehydrogenases and NADH- and NADPH tetrazolium reductases during latter stages of degeneration.

Keywords. Atresia; dehydrogenase; follicle; granulosa; lipids; NADH- and NADPH-tetrazolium reductases; thecal layer; *Tatera indica*.

1. Introduction

Mammalian follicular wall consisting of thecal and granulosa layers undergoes conspicuous morphological, histochemical and biochemical changes during the normal process of follicular growth (Guraya 1973a; Bjersing 1978) as well as during atresia (Guraya 1973a,b; Byskov 1978). Theca interna at all stages of follicular growth shows ultrastructural and histochemical features of a typical steroidogenic tissue (Guraya 1973a, 1980; Bjersing 1978) and seem to form a major source of follicular steroid hormones (Fortune and Armstrong 1977; Bohr *et al* 1980). The granulosa cells of growing follicles do not show such characteristics related to steroidogenesis, but develop some degree of luteinization in the large preovulatory follicles (see Bjersing 1978). These studies indicate that the steroidogenic character of granulosa cells vary in growing and mature follicles and also under different physiological situations (Pupkin *et al* 1966).

Though there are a number of reports dealing with distribution of lipids and enzymes related to steroidogenesis and the general ovarian metabolism in different ovarian compartments (Hoyer 1980; Guraya 1980), only a few deal with their changes during stages of follicular growth and atresia, particularly in seasonally breeding wild species of mammals. Remarkable changes occur in the ovary of wild rodents during different seasons; winter ovary is usually inactive and reduced while that of summer is active (Guraya and Gupta 1979). Therefore, the active summer ovaries of the Indian gerbil *Tatera indica* were subjected to various histochemical tests for lipids

and enzymes related to carbohydrate metabolism and steroidogenesis to reveal changes in follicular wall during growth and atresia.

2. Materials and methods

The ovaries of sexually mature Indian gerbil rat *Tatera indica* Hardwicke were used. The rats were collected by trapping from the field areas of Ludhiana from March to July the period of their breeding activity. Fresh-frozen cryostat sections (12-14 μ m thickness) were placed on coverslips, thawed and kept at room temperature for 10-15 min before incubation in different media for the localization of NADH- and

Table 1. Histochemical localization of lipids, tetrazolium reductases and dehydrogenases in the follicular wall during growth and atresia of follicles and interstitial gland tissue in the ovary of *Tatera indica*.

		Stage I		Stage II		Stage III		Stage IV		Stage V		Interstitial gland tissues
		GC	TL	GC	TL	GC	TL	GC	TL	GC	TL	
Sudan black B in 70% ethanol (Pearse 1968)	NF	1	2	1	3	1	4	1	4	1		5
	AF	?	2	2	3	2	4	4	4	4		
Schultz test (Pearse 1968)	NF	0	1	0	2	0	2	0	3	0		4
	AF	0	1	0	2	1	2	1	3	1		
NADH-tetrazolium reductase	NF	2	3	3	4	3	4	3	4	4		5
	AF	?	4	1	5	1	5	1	5	1		
NADPH-tetrazolium reductase	NF	2	2	2	3	3	3	3	4	3		4
	AF	?	3	1	4	1	4	1	4	1		
$\Delta^5\beta$ -HSDH (DHA)	NF	0	2	0	3	0	3	2	4	2		4
	AF	0	2	0	3	0	3	0	4	2		
$\Delta^5\beta$ -HSDH (pregnenlone)	NF	0	2	0	3	0	3	0	4	0		4
	AF	0	2	0	3	0	3	0	4	0		
17 α -HSDH (17 α -hydroxy-gesterone)	NF	0	1	0	1	0	2	0	3	1		2
	AF	0	1	0	1	0	1	0	1	0		
Succinic dehydrogenase	NF	2	3	2	3	3	4	4	4	4		5
	AF	0	3	1	3	2	3	1	4	1		
Lactate dehydrogenase	NF	2	1	2	2	3	1	4	3	4		3
	AF	?	1	1	2	1	3	1	3	1		

AF, atretic follicle; GC, granulosa cells; NF, normal follicle; TL, thecal layer; ? not observed; 0, no activity; $\Delta^5\beta$ -HSDH, $\Delta^5\beta$ -hydroxysteroid dehydrogenase; 17 α -HSDH, 17 α -hydroxysteroid dehydrogenase; 1 to 5, indicating increasing intensity of reaction.

NADPH- tetrazolium reductases, hydroxysteroid dehydrogenases and succinate- and lactate dehydrogenases (table 1). For the localization of enzymes tetrazolium salt procedures along with controls were followed (Lojda *et al* 1979). Some sections of each batch of the ovarian material were fixed in formaldehyde-calcium and aqueous Bouins fluid. These were stained with Sudan black B (SBB) and Schultz test (Pearse 1968) for localization of general lipids and cholesterol respectively. The amounts of lipids, cholesterol and diformazan precipitates in the sections were evaluated on a 0 to 5 scale (table 1) by a single observer.

3. Results

The histochemical localization of lipids and enzymes has been studied in the following stages of follicular growth and in atretic follicles of the corresponding stages.

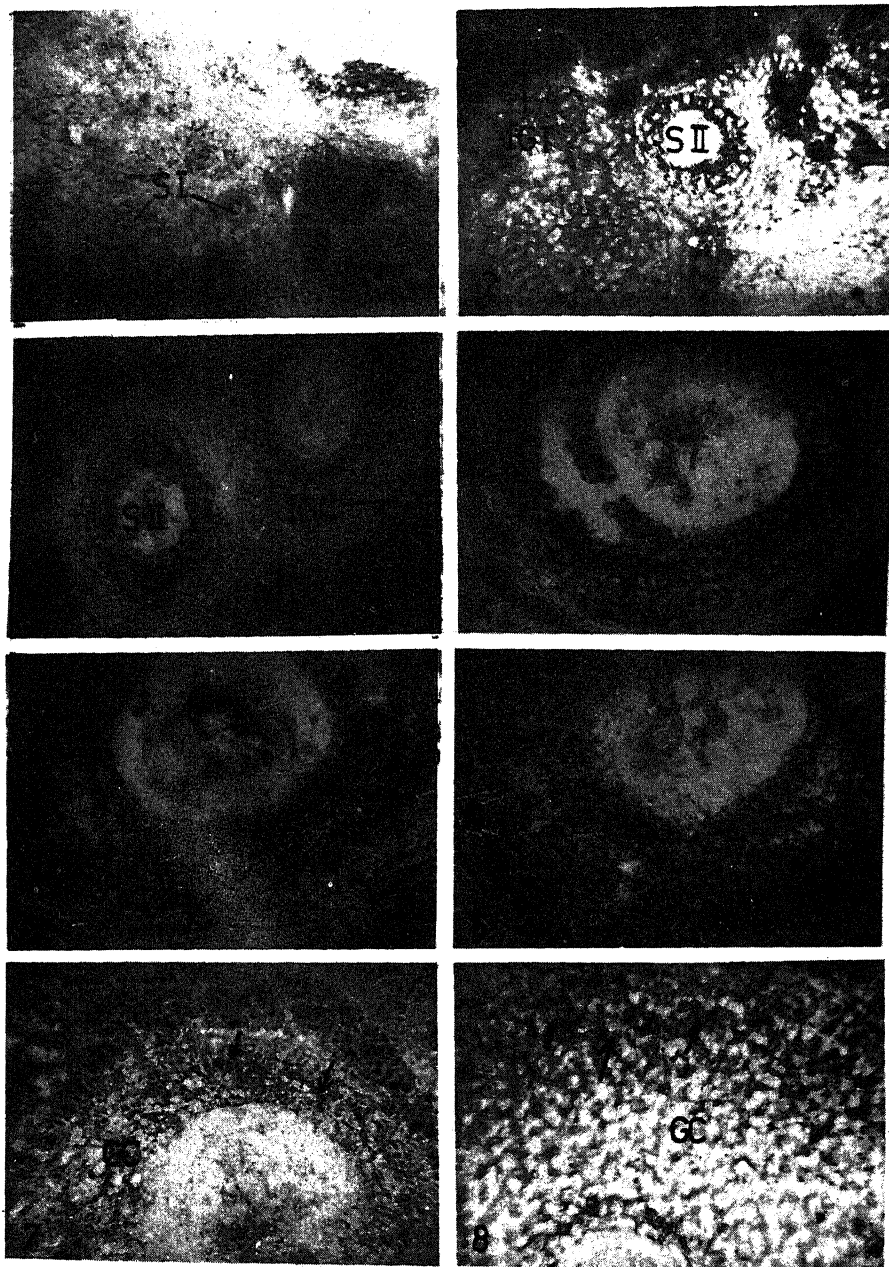
- Stage I — Preantral follicles with few to many granulosa cells arranged in a single layer.
- Stage II — Preantral follicles with 2 to 3 layers of granulosa cells.
- Stage III — Preantral follicles with 4 and more layers of granulosa cells.
- Stage IV — Follicles with incipient antral formation as indicated by series of isolated lacunae.
- Stage V — Antral follicles with a single large antrum.

The results of histochemical reactions in the thecal and granulosa layers of normal and atretic follicles of different stages are summarized in table 1. For the comparison of histochemical reactions (presented in table 1) between the follicular wall of normal and atretic follicles, the atretic follicles showing some degree of morphological identity of their follicular layers were considered. Changes during latter stages of degeneration were more drastic as the granulosa cells disappear and are replaced by lipid accumulations (figures 15 and 16).

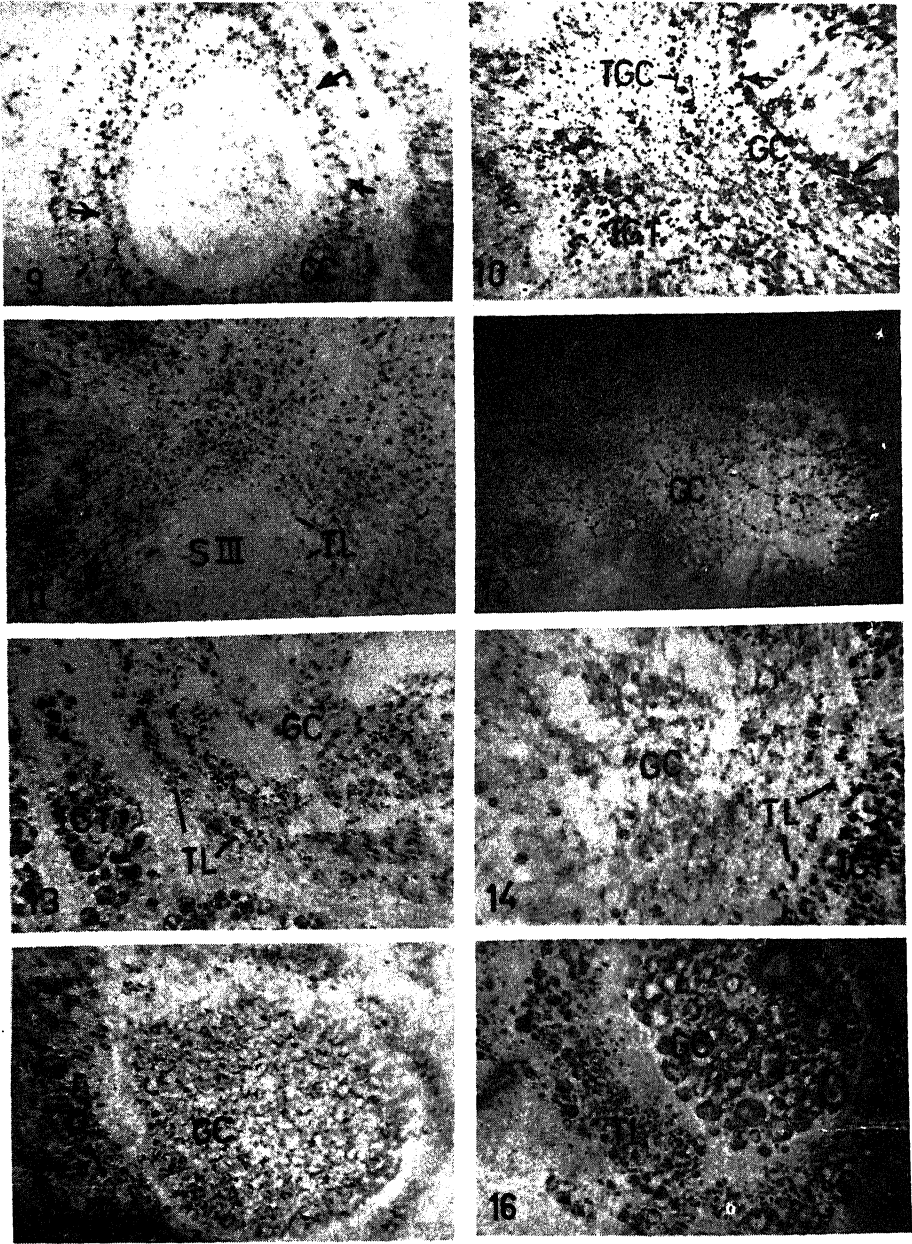
3.1 Tetrazolium reductases

Both NADH- and NADPH- tetrazolium reductases show similar pattern of staining reactions in normal and atretic follicles of different stages, but the latter show relatively lesser activity. The thecal layer continues to show similar staining reaction in all stages of follicular growth but a notable increase occurs in the atretic follicles of corresponding stages (table 1).

A gradual increase in the activity of tetrazolium reductases occurs during the organization of single-layered granulosa layer as well as during subsequent stages of follicular growth (figures 1 to 4). The inner layers of granulosa in stage II normal follicles contain relatively more activity than its outer layers (figure 3). During further growth, some stage IV follicles show more NADPH- tetrazolium reductase activity in the middle layers of their granulosa (figure 4) while others show both NADH- (figure 5) and NADPH- tetrazolium reductases (figure 6) in the outer layers. In contrast to the thecal layer, the granulosa shows a marked decrease in enzyme activities in atretic follicles which disappear completely during latter stages of atresia.



Figures 1-8 (See captions on p.128)



Figures 9-16 (See captions on p. 128)

3.2 Succinate and lactate dehydrogenases

With follicular growth, succinate- and lactate- dehydrogenases increase both in the thecal and granulosa layers. The outer layers of granulosa cells in normal growing follicles show more activity than the inner layers (figures 7 and 8). LDH tends to show more activity in the middle granulosa layers of some follicles (figure 9) than in their innermost layer (figure 10). Atresia greatly decreased the activity of SDH in granulosa cells (figure 14) with little or no change in LDH at an early stage of degeneration (figure 13). During latter stages of degeneration weak LDH activity occurs in the granulosa cells of atretic follicle (figure 13) but SDH disappears completely (figure 14). Atresia has no effect on the SDH and LDH activities in the thecal layers.

3.3 Steroid dehydrogenases

Theca interna of normal follicles shows continuous increase in enzyme activities of Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSDH) during follicular growth. Granulosa cells in large follicles of stages IV and V show some activity of Δ^5 -3 β -HSDH when dihydroepiandrosterone (DHA) was used as substrate but no visible activity appears with pregnenolone (table 1). Granulosa cells in earlier stages of follicular growth are devoid of Δ^5 -3 β -HSDH (figures 11 and 12). Weak activities of Δ^5 -3 β -HSDH and 17 α -hydroxysteroid dehydrogenase (17 α -HSDH) appear in granulosa cells of stage V follicle (figure 12) and they disappear during latter stages of atresia. Atresia has no effect on the Δ^5 -3 β -HSDH activity in the thecal layer. Weak reaction of 17 α -HSDH in thecal layer of different stages persist even after the degeneration of granulosa but in stage V follicles a decrease occurs in its activity.

3.4 Lipids

Few sudanophilic granules, which fail to stain with Schultz's technique for cholesterol, are seen in single-layered granulosa cells of stage I follicles. The granulosa cells do not show any appreciable change in the amount of lipids during follicular growth except that increasing lipid deposition occurs in antral follicles of stage V, especially in the peripheral layers of their granulosa cells lying adjacent to the basement membrane. As compared to the earlier stages of follicular growth, some increase in the amount of lipid granules is also seen in the thecal layer. After its differentiation, the theca interna continues to stain for cholesterol-positive lipids which are present in the thecal gland cells; a progressive increase in their amount is seen during the growth of follicles. Follicular atresia does not affect the histochemical reactions for lipids in the thecal layer but the granulosa cells of atretic follicles of all stages show a marked increase in lipids. The lipids begin to accumulate in granulosa cells at the initial stages of atresia and as the degenerative process advances the amount of lipids is increased to form highly sudanophilic accumulations of large size.

4. Discussion

The comparison of the histochemical features of follicular wall in normal and atretic follicles of corresponding stages in the ovary of the Indian gerbil *Tatera indica* has revealed conspicuous changes in the amount of lipids and activities of tetrazolium reductases, succinate- and lactate-dehydrogenases. In contrast to previous observations

on rat, mouse and guinea pig (Motta 1972) the granulosa cells of normal and atretic follicles of different stages, except of Graafian follicles in *T. indica*, are devoid of any histochemically detectable change in the activities of Δ^5 - 3β -HSDH and 17α -HSDH.

Atresia of follicles of all stages results in increased accumulation of lipids in the granulosa cells as reported for other species (Guraya 1973b; Guraya and Greenwald 1964a,b). Decreased activities of tetrazolium reductases, lactate- and succinate-dehydrogenases in granulosa cells suggest that atresia affects the mitochondrial functions and reoxidation of NADH and NADPH coenzymes. This is also supported by the structural changes in the mitochondria of granulosa cells of atretic follicles in the mammalian ovary (Albertini 1980; Dimino and Campbell 1980). Though the granulosa cells accumulate cholesterol-positive lipids in atretic follicles the absence of steroid dehydrogenases activities in the granulosa cells of both growing and atretic follicles of corresponding stages (from stages I to IV) suggests that alterations in the activity of tetrazolium reductases and other mitochondrial enzymes cannot be related to the effect of atresia on the development of luteinization process during the phase of follicular growth. However, the luteinization of granulosa cells seems to occur in the Graafian follicles as evidenced by the development of lipid accumulation and some increase in the activities of tetrazolium reductases and steroid dehydrogenases. Differences between histochemical features of different layers of granulosa cells of multilamellar follicles as shown previously in hamster (Knigge and Leatham 1956) and rabbit (Guraya and Greenwald 1964a) may reflect variable responses of different layers just before the onset of atresia or indicate their variable character during normal growth of the follicles.

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Figures 1-8. 1. A portion of the ovary of *Tatera indica* showing moderate activity of NADH tetrazolium reductase in granulosa cells of stage I (SI) follicle 2. Showing activity of NADPH tetrazolium reductase in granulosa cells arranged in a single layer in stage II (SII) follicle. Note that interstitial gland tissue (IGT) shows strong activity of this enzyme 3. Stage II normal follicles showing relatively more activity of NADH tetrazolium reductase in the inner layers of granulosa cells. Thecal gland cells (TGC) and interstitial gland tissue (IGT) show strong activity of the enzyme 4. A portion of stage IV follicle showing more activity of NADPH tetrazolium reductase in the middle layers of granulosa cells (arrow). The oocyte contains some non-specific activity 5. NADH tetrazolium reductase in the outer layers of granulosa cells (arrow) 6. NADPH tetrazolium reductase in the outer layers of granulosa cells. 7. Lactate dehydrogenase (LDH) enzyme in outer layers of granulosa cells (GC) 8. Succinic dehydrogenase enzyme in the outer layers of GC ($\times 400$).

Figures 9-16. 9. Stage IV follicle showing relatively more activity (arrows) of LDH in the inner and middle layers of GC. The outer most layer which is below the basement membrane contain weak activity ($\times 100$). 10. Showing similar intensity of staining of enzyme activity of SDH in the IGT and TGC and inner layers of GC of stage III follicle ($\times 100$). 11. Showing absence of Δ^5 - 3β -hydroxysteroid dehydrogenase (Δ^5 - 3β -HSDH) enzyme activity in granulosa layers of stage III follicle and is present in the thecal layers (TL) and the adjoining stroma ($\times 100$) 12. Showing moderate activity of Δ^5 - 3β -HSDH in the GC and TL of stage V follicle and interstitial gland tissue ($\times 400$) 13. A portion of atretic follicle of stage IV showing that no change occur in enzyme activity of LDH in GC and TCG. Note disorganization of layers of granulosa cells ($\times 400$). 14. A portion of stage IV atretic follicle showing decreased activity of SDH in GC and no change in TL and IGT. The dense colour in the granulosa cells is the non-specific staining in their nuclei ($\times 400$). 15. Late stage atretic follicle of stage IV showing decreased activity of LDH in GC and no effect of atresia on enzyme activity in TL ($\times 100$). 16. Late stage atretic follicle of stage IV showing absence of SDH activity in GC and no effect of atresia on enzyme activity in TL ($\times 100$).

Patterns of rodent pest infestation in relation to rainfed and irrigated crop systems in Rajasthan

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Abstract. A field study on the relationship between rodent fauna and crop ecosystem of the Indian Desert and its two main systems was undertaken in 5 villages and 7 dhanis near Jodhpur. Out of twelve rodent species, *Meriones hurrianae* had a highest relative abundance followed by *Tatera indica indica*, and *Rattus meltda pallidior*. Rodent fauna in the winter irrigated crops and fields was more diversified (11 species) than rainfed summer crops and fields (10 species). *M. hurrianae* was a predominant species in rainfed crop fields while, *T. indica* along with *M. hurrianae* was a codominant species in irrigated winter crops. *Rattus gleadowi* and *Vandeleuria oleracea* from summer crop fields and *Mus cervicolor phillipsi* in irrigated crop fields, were altogether absent. Relative abundance of *Funambulus pennanti*, *R. meltda*, *Mus booduga*, *Golunda ellioti gujerati* and *T. indica* increased from rainfed to irrigated crop systems, whereas, reverse trend was witnessed for *Rattus rattus rufescens*, *M. hurrianae* and two *Gerbillus* spp. (*nanus* and *gleadowi*). Occurrence patterns of different rodent species in the crop ecosystem of Indian desert as a whole, and in irrigated and rainfed crop systems in particular have been critically discussed keeping in view their ecological, physiological, behavioural and biological patterns.

Keywords. Indian desert; infestation patterns; rodent biomass.

1. Introduction

Rodents, as one of the major vertebrate pests of crops at their all growth stages, from sowing to storage through threshing, cause severe losses to overall grain production in the Rajasthan desert, the region already having dangers of crop failures and drought due to insufficient rainfall and its erratic patterns (Advani 1982). The rodent damage to several vegetable (Advani and Mathur 1982) and wheat crops (Advani 1982; Advani *et al* 1982) has been evaluated as 19.81 and 18.74% respectively.

Owing to seriousness of rodent pest problem in the crop ecosystem of Rajasthan desert, a four year (1978-1981) study was undertaken to find out rodent-crop relationship. Coinciding two rainfall periods; summer (June-August) and winter (December-February), summer (rainfed) and rabi winter (irrigated) respectively. The present paper embodies relationship between these two crop systems and species composition, relative abundance and biomass of rodents prevalent.

2. Crop habitat

Studies were undertaken in about 2000 hectare cropped area of five villages and seven dhanis (few houses encircled by cropfields and rangelands) near Jodhpur (26° 18'N, 73° 01'E), a representative desert district in Rajasthan. During June to

September among kharif (rainfed) crops, *bajra* (*Pennisetum typhoides*—a millet) is grown on about 90% of the land receiving adequate rainfall for its sowing and further growth. In addition to this some legumes and vegetables (like brinjal) are also cultivated as summer rainfed crops. In the winter rainfall period (December to February), with most of the area under wheat (*Triticum aestivum*) crop, several types of vegetables (mainly chillies, tomato, cabbage, cauliflower and other cucurbits) are harvested.

The plant and grass species in the rangelands and sandy habitat around cropfields which support rodents for food, shelter and burrowing during periods of food scarcity (April to June) are—*Prosopis cineraria*, *Acacia tortilis*, *Zizyphus nummularia*, *Calligonum polygonoides*, *Aerva tomentosa*, *Calotropis procera*, *Tephrosia purpurea*, *Lasiurus indicus*, *Cenchrus ciliaris*, *C. setigerus*, *C. biflorus*, *Aristida* spp. and *Dichanthium annulatum* (Prakash 1974). The habitat is characterized by high temperatures and low and erratic rainfalls. The annual rainfall varies from 88 to 425 mm (Anon. 1969), the number of rainy days being 2.7 to 28.5 per annum. The highest and lowest temperatures recorded in the past ten years are 49°C and -2°C, with annual average minimum and maximum temperatures varying from 16.4°C to 22.2°C and 32.2°C to 34.6°C respectively.

3. Materials and methods

Following international standard technology (Barnett and Prakash 1975), the trap indices (rodents/100 traps/24 hr), species composition, relative abundance and biomass (g/hectare) of rodents were evaluated by installing trap lines of snap (museum) traps in a grid manner in both types of crop seasons. For kharif (rainfed) crop the cropfields with bajra etc. were selected, while for rabi (rainfed) crop, traps were operated in wheat, vegetables and oilseed crop fields. Each trap line in each crop system was having 260 snap traps fixed to a wooden peg inserted deep into soil. The distance between the two traps was 10-15 m keeping in view home ranges of prevalent rodent species of Rajasthan desert.

After evaluating general infestation patterns of rodents in crop ecosystem as a whole, the sample sizes of individual rodent species were distributed between summer (rainfed) and winter (irrigated) crops to find out seasonal and cropwise species composition and relative occurrence. The trap lines were checked after every 4 hr and the dead snap trapped rodents were removed. During this checking traps were also rebaited. On the basis of morphological characteristics, rodents were identified after Ellerman (1961) and then preserved, registered and deposited in the museum of Animal Ecology and Rodent Project at Central Arid Zone Research Institute, Jodhpur.

4. Results

Twelve species of rodents were collected from the crop fields having summer (rainfed) and winter (irrigated) crops (table 1). Spread over three families and eight genera, one species (8.33%) belonged to sciurids, seven species (58.33%) belonged to murids while, four species (33.33%) represented by gerbils (79.44%) predominated over murids (18.18%) and sciurids (2.30%) groups of rodents. Among gerbils as well as all twelve rodent species, Indian desert gerbil, *Meriones hurrianus* was a predominant species, followed by another gerbil, *Tatera indica indica* and then murid, Soft-furred

Table 1. Rodent infestation patterns in the crop ecosystem of the Indian desert.

Species	N=	% relative abundance			% distribution	
		All crops	Summer crop	Winter crop	Summer crop	Winter crop
<i>Funambulus pennanti</i>	6	2.32	0.81	3.67	16.6	83.3
<i>Rattus leadowi</i>	1	0.38	0.0	0.73	0.0	100.0
<i>Rattus rattus rufescens</i>	5	1.93	2.45	1.47	60.0	40.0
<i>Rattus meltada pallidior</i>	24	9.30	6.55	11.76	33.3	66.6
<i>Mus cervicolor phillipsi</i>	2	0.77	1.63	0.0	100.0	0.0
<i>Mus booduga</i>	4	1.55	0.81	2.20	25.0	75.0
<i>Golunda ellioti gujerati</i>	10	3.87	1.63	5.88	20.0	80.0
<i>Vandeleuria oleracea</i>	1	0.38	0.0	0.73	0.0	100.0
<i>Meriones hurrianae</i>	116	44.96	55.73	35.29	58.6	41.3
<i>Tatera indica</i>	85	32.94	28.68	36.76	41.2	58.8
<i>Gerbillus nanus indus</i>	2	0.77	0.81	0.73	50.0	50.0
<i>Gerbillus leadowi</i>	2	0.77	0.81	0.73	50.0	50.0

Table 2. Fluctuations in rodent biomass (g/hectare) in the fields of two main crop systems of Indian desert.

Crops systems	Years (biomass g/hectare)			
	1978	1979	1980	1981
Summer (rainfed crop fields)	1836	1730	1961	1825
Winter (irrigated crop fields)	2112	1975	2324	2024

field rat, *Rattus meltada pallidior*. Occurrence of other nine species varied from moderate to very low numbers.

When total sample sizes of field rodents were segregated between two major crop systems, percent relative abundance of *M. hurrianae* was found to be highest in the summer (rainfed) crop fields. Whereas, in winter (irrigated) conditions, *M. hurrianae* and *T. indica* were observed as two co-predominant species in the rodent fauna. Relative percent occurrence of Indian palm squirrel, *Funambulus pennanti*, *R. meltada*, Field mouse, *Mus booduga*, Bush rat, *Golunda ellioti gujerati* and *T. indica* increased considerably from summer to winter crop fields. Whereas, reverse trend was witnessed in case of House rat, *Rattus rattus rufescens*, *M. hurrianae* and two other smaller gerbil species, *Gerbillus nanus indus* and *G. leadowi*, though not much pronounced in case of latter two species.

Regarding percent distribution of total populations of individual rodent species trapped, *F. pennanti*, *R. meltada*, *M. booduga*, *G. ellioti* and *T. indica* preferred to

infest irrigated winter crops in preponderance. On the other hand, *R. rattus* and *M. hurrianae* infested rainfed crop fields and crops in higher numbers to irrigated ones. Whereas, the populations of *R. gleadowi*, *G. nanus* and *G. gleadowi* (all smaller species) were distributed equally between two crop systems.

In summer as well as winter crops, the rodent biomass was encountered in relatively lower magnitude in 1979 as compared to other years in the same crop systems (table 2). However, during 1980, the rodent biomass was the highest in both crops. In general with higher rodent biomass in winter crops (to summer rainfed crops), the annual fluctuations showed more or less a steady pattern in case of individual crop systems.

5. Discussion

Occurrence of a diversified rodent fauna in the crop ecosystem of the Indian desert is due to availability of diversified habitats, different crops, sandy and range land area suitable for rodents to burrow. Moreover, as the Indian desert is located at cross-roads of the Oriental, Palearctic and Ethiopian Zoo-geographical regions from where cross-migration and establishment of different rodent species in this biome is quite expected (Prakash 1963). Moreover, all the gerbil species, which constitute about $\frac{3}{4}$ th of crop rodent fauna, have all ecophysiological adaptations, particularly resistance to food and water scarcity (Advani and Prakash 1980), salt tolerance (Ghosh and Gaur 1966) and feeding adaptations (Prakash 1962). The predominant gerbil species, *Meriones hurrianae* possess all behavioural (Advani 1981a), biological (Prakash 1971) and eco-physiological adaptations for its survival, reproduction and maintenance of higher populations. The codominant gerbil species, *T. indica* also breeds throughout the year and has also higher annual productivity rate (Prakash 1971) than all other rodent species.

As the summer (rainfed) crop fields are mostly established in the rangelands and sandy plains, where *M. hurrianae* is a predominant species, it infests crop fields (mostly millet, bajra, *P. typhoides*) in relatively higher percentages. However, in the winter irrigated crop fields with wheat, chilli and most of the vegetable crops, it co-shares predominance with *T. indica* which is a major pest of wheat (Advani *et al* 1982) and vegetables (Advani and Mathur 1982). In the irrigated crop fields, *T. indica* as well as *R. meltada pallidior* which require some moist soil or surrounding mud walls around the crop fields to burrow, justify their existence in winter crop in more numbers. *R. meltada* population can also depend upon stored wheat in hutments in absence of crop in field (Rana and Advani 1981). *G. ellioti*, a pest of wheat threshing floors (Advani 1981 b,c) is also likewise collected in moderate abundance in irrigated over-rainfed crops. Dependence of squirrel over fruits, vegetables etc., for food and surrounding large neem, banyan (*ficus*) and fruit trees for nesting (Banerji 1955), regulate their occurrence in crop fields under irrigation in winter season. Relative occurrence of higher rodent biomass during the year 1980 in both crop ecosystems, is probably due to average good rainfall during that year which had an ultimate impact on better crop production, essential to support higher rodent biomass.

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Dietary water balance in a tropical insect

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Abstract. Dietary water intake of the tropical grasshopper *Poeciloceris pictus* fed *ad libitum* from hatching to death on the milkweed *Calotropis gigantea* averaged 85 or 51 g at 26°C and 171 or 78 g at 36°C for a female and male respectively. As much as 54 or 96 g dietary water is lost via faeces by a female at 26 or 36°C; the corresponding values for a male was 37.5 and 36.2 g. A male or female carried at death about 9 or 6% of the respective dietary water absorbed at 26 or 36°C. Water loss via exuvia amounted to 26 mg in all the series. Due to oviposition 4.8 or 7.4% of the total water absorbed was lost by a female at 26 or 36°C. There exist a very close relationship between the efficiencies of matter assimilation and water absorption. The high dietary water intake and absorption rates reported for the tropical lepidopteran *Danaus chrysippus* are correlated with the accumulation of water to meet the requirements during the nonfeeding pupal stage.

Keywords. Dietary water; *Poeciloceris pictus*; *Calotropis gigantea*; exuvia; transpiration; *Danus chrysippus*.

1. Introduction

Water is an important factor which controls the limits of distribution of terrestrial animals (Allee *et al* 1949). Insects form an important component both in terms of number or biomass and functional unit in terrestrial communities (Engelmann 1966). Experimental determinations of energy budget are known to offer most important clues in regard to the success of aquatic organisms (Kinne 1960; Brett *et al* 1969); data on the energy budget supplemented with water budget, are required to know the important clues with regard to the success of a terrestrial animal (Michael *et al* 1971; Reese and Beck 1978; Scriber 1977, 1979; Scriber and Slansky 1981).

Data on energy budget of a number of terrestrial insects (from hatching to death) have recently been reported (Delvi and Pandian 1971; Delvi 1972; Hiratsuka 1920; Schroeder 1971; Scriber and Slansky 1981; Delvi and Premaleela 1983). Some have considered the water balance of insects taking into account one or two physiological stages and instars (Pandian *et al* 1978; Baker and Lloyd 1970). Information on the water balance of an insect from hatching to death is scanty although the means and the extent of intake and loss of water are many, complex, interrelated and continuously oscillating.

Some insects are known to obtain atmospheric water through the integument (Bodine 1921; Lugwig 1937; Beament 1964) and in a few cases *via* the cloacal ends (Beament 1961) or the spiracles (Buxton 1932). On the other hand, body water may also be lost to the atmosphere through the integument or *via* spiracles during expiration

(Uvarov 1966). Water losses through spiracles and cuticle are easy to separate and both are usually considered together as transpiration (Uvarov 1966). The dynamic aspects of these physiological processes of absorption of atmospheric water and transpiration of body water through the integument have been dealt with by many workers (Wigglesworth 1957; Edney 1957, 1967; Eberling 1964; Cloudsley-Thompson 1962; Beament 1964 and Bursell 1964).

Relatively more water is taken orally; many insects are known to drink (Barton-Browne 1964), but others particularly stored-product pests and perhaps many leaf feeders get all or most of the water from the food ingested (Waldbauer 1968; Millot and Fontaine 1937; Lee 1961). Another possible internal source of metabolic water is *via* fat oxidation; 100 g fat upon oxidation is known to yield 107 g of metabolic water, 100 g carbohydrate yields 56 g water and 100 g protein yields 41 g water (Baldwin 1964). Such metabolic water is of great significance in large fluid-feeders (Schmidt-Nielsen and Schmidt-Nielsen 1953). However, its importance in smaller insects, especially those feeding on carbohydrate rich leaves is less (Wharton and Arlian 1972). Perhaps, *P. pictus* meets a major fraction of the required water from the ingested leaves of the milkweed *Calotropis gigantea*. Body water is also lost through faeces (which includes urine), exuvia egg and associated products and other secretions (eg. silk etc.). The quantitative aspects of water gain *via* food and loss *via* excretory and faecal products, as well as the mechanisms regulating them have not been studied adequately and the importance of such data to understand adaptive mechanisms in arthropods inhabiting different habitats has been emphasised (Vernberg and Vernberg 1971; Pandian *et al* 1978). This paper reports the dietary water budget of a tropical insect *Poeciloceris pictus* (Family: Pyrgomorphidae) reared from hatching to death at two different temperature levels.

2. Material and methods

The grasshopper *P. pictus* (15 to 25 individuals in each series) was reared in the laboratory feeding on the fresh leaves of *C. gigantea* from hatching to death at room temperatures of $26 \pm 2^\circ\text{C}$, $70 \pm 20\%$ r.h. and 8 hr photoperiod and $36 \pm 2^\circ\text{C}$, $60 \pm 10\%$ r.h. and 12 hr photoperiod. Fluctuations in relative humidity were in the range of $\pm 10\%$ in a day and ranged from 70 to 90% at any one time during a year. Insects transfer water into their blood from air at relative humidity as low as 70% (Beament 1964). Since the experiments at room temperature of 26°C were performed at an ambient r.h. of 70%, considerable amount of atmospheric water could have been transferred into *P. pictus*; however, this was not estimated separately. The water absorbed from the atmosphere was included in the changes in the body weight of *P. pictus*. At 36°C , the ambient relative humidity was 60% and hence the transfer of such water into *P. pictus* may be very limited.

Fresh leaves of the milkweed *C. gigantea* offered as test food for *P. pictus* were always culled from the same large plant growing in the University campus, cut and offered, following the standard procedure (Waldbauer 1968).

The test individuals normally feed immediately after the food is offered; the satiation time (the time from the commencement of feeding to voluntary cessation, Brett 1971), lasted 3 to 5 min in the first few instars and 8 to 13 min in subsequent instars and adults. During the first 30 min, the water loss owing to evaporation from the leaves in the terraria without insects averaged $4.3 \pm 0.4\%$ at 26°C and $6 \pm 3.1\%$ at

36°C of the total initial leaves given. Since half the food was consumed before 1/3 of the respective period, the water loss from the leaf might have been lesser than that estimated above; hence 1/3 of the estimated value *i.e.* 1.1% at 26°C and 1.5% at 36°C may be taken to assess experimental error in measuring the intake of water through food plant.

The water content and chemical composition of plants vary as function of age and season (Golley 1961). The leaves of *C. gigantea* are thick, leathery and contain an average water content of $86 \pm 1.5\%$. The variations in the initial water content of the leaves and the changes in the water content owing to transpiration and evaporation in the leaves during its presence in the terraria amount to 3.5%.

The faecal pellets produced by sample individuals of *P. pictus* belonging to different life stages and sex reared at 26 and 36°C were collected as they were being defecated and their respective water content estimated by drying in an oven maintained at 105°C, until weight constancy. Water content of the faeces at 26 or 36°C varied from 77 to 89%. Since the mean difference between the respective mean water contents of leaf and faeces was only 2.5%, water content of faeces and leaf was considered to be the same (see also for *P. pictus* Delvi and Pandian 1972, for *Schistocerca gregaria*, Phillips 1964). Dietary water retained in the body during different life stages was estimated by sacrificing sample individuals reared under the same conditions in the laboratory. Mean water content values of these individuals are presented as functions of life stage, sex and temperature in table 1. The SD value exceeded 5 only in very few cases. The average of all the SD amounted to 3 which is 4.1% (coefficient of variation) of the mean obtained for water content of different life stages. On the whole, the experimental error in estimating dietary water intake, loss *via* faeces and retention in the body may not exceed 8.9%.

Table 1. Mean water content of the grasshopper *P. pictus* fed *ad libitum* on *C. gigantea* at $26 \pm 2^\circ\text{C}$, $70 \pm 20\%$ r.h. and at $36 \pm 2^\circ\text{C}$, $60 \pm 10\%$ r.h.

	Female		Male	
	26°C	36°C	26°C	36°C
Hopper (Instar)				
I	79.7 ± 0.03	79.7 ± 0.03	79.7 ± 0.03	79.7 ± 0.03
II	77.3 ± 1.66	72.5 ± 1.7	75.6 ± 2.8	72.8 ± 5.1
III	80.7 ± 2.3	74.5 ± 2.1	75.9 ± 4.2	71.0 ± 1.2
IV	75.2 ± 3.4	75.0 ± 3.1	74.2 ± 4.6	76.0 ± 0.8
V	77.6 ± 4.2	73.8 ± 2.0	74.7 ± 4.8	72.9 ± 4.5
VI	77.1 ± 2.1	77.97 ± 4.9	74.8 ± 1.2	74.2 ± 3.8
Adult				
I fortnight	76.2 ± 2.0	73.8 ± 5.4	74.6 ± 4.0	73.9 ± 3.1
Before oviposition	67.8 ± 3.1	—	74.9 ± 3.5	—
After oviposition	60.2 ± 2.9	—	66.6 ± 5.4	—
At death	58.3 ± 5.3	65.3 ± 3.1	60.4 ± 6.3	64.1 ± 4.8

Each value represents the average of 10 to 25 animals, which were dried immediately after moulting.

3. Results and discussion

Mean values obtained for total dietary water intake of *P. pictus* as functions of life stage, sex and temperature are presented in tables 2 and 3. In general, total dietary water intake increased from the first instar to a maximum in the sixth instar (e.g. 17.5 g for females at 26°C, table 2) or in the first fortnight of the adult life (e.g. 41.2 g for females at 36°C, table 3). Mean total dietary intake amounted to 84.85 or 51.35 g for a female or male reared at 26°C, while it was as much as 170.8 g for a female and 78.1 g for a male reared at 36°C.

As the life stages of *P. pictus* included different days (see tables 2 to 7; column 2), dietary water intake was calculated in mg/day. Daily dietary water intake increased from about 12 or 36 mg in the first instar at 26 or 36°C to a maximum of 684 or 351 mg for a female and male respectively in the first fortnight of adult life at 26°C and to 2748 mg in the first fortnight of adult female or to 1151 mg in the sixth instar male at 36°C. Subsequently, the daily water intake decreased in all cases.

The mean dietary water intake rate was highest in the second instar female (558.8 mg/g/day) and male (771.7 mg/g/day) at 26°C, whereas it was over 1200 mg/g/day in the second instar male and fourth instar female at 36°C (table 3). In most cases, dietary water intake rate decreased with increasing body weight and age of *P. pictus*.

As indicated earlier, dietary water loss may be *via* (i) faeces, (ii) exuvia, (iii) reproductive products, (iv) from the grasshopper at death and (v) transpiration. Since the first 4 and total dietary water intake in *P. pictus* have been estimated, the dietary water loss *via* transpiration could be calculated. Mean total water loss *via*

Table 2. Dietary water intake in *P. pictus* fed *ad libitum* on *C. gigantea* at 26 ± 2°C, 70 ± 20% r.h., and 8 hr/day photoperiod.

Life stage stage	Average duration (day)		Middle body weight (mg)		Mean total water intake (g/instar)		Mean daily water intake (mg)		Water intake rate (mg/g/day)	
Hopper (Instar)	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
I	30	31	35.6	33.3	0.378	0.357	12.6	11.5	353.9	345.9
II	16	15	102.0	80.6	0.915	0.932	57.2	62.2	558.8	771.7
III	19	16	207.0	181.8	1.613	1.377	84.8	86.1	410.1	474.1
IV	21	24	393.4	376.0	3.091	2.419	147.2	100.8	374.2	268.1
V	27	32	912.1	797.5	8.114	6.989	300.5	218.4	329.5	273.9
VI	49	40	2011.0	1483.7	17.447	14.801	356.1	370.0	177.1	236.0
Adult (fortnight)										
I	15	15	3151.0	1984.0	10.265	5.258	684.3	350.6	217.2	176.7
II	15	15	3832.0	2240.0	8.576	5.090	571.7	339.4	149.2	151.5
III	15	15	4133.0	2270.0	8.274	3.763	551.6	250.9	133.5	110.5
IV	15	15	4328.0	2610.0	7.661	3.108	510.7	207.2	182.4	79.4
V	15	15	4615.0	2630.0	7.207	2.671	480.5	178.1	104.1	67.7
VI	15	15	4939.0	2435.0	4.906	2.293	327.1	152.9	66.2	62.8
VII	15	15	4939.0	2148.0	3.200	2.293	213.4	152.9	42.8	62.8
VIII	15	—	4939.0	—	3.200	—	213.4	—	42.8	—

Table 3. Dietary water intake in *P. pictus* fed *ad libitum* on *C. gigantea* at $36 \pm 2^\circ\text{C}$, $60 \pm 10\%$ of r.h. and 12 hr/day photoperiod.

Life stage	Average duration (day)		Middle body weight (mg)		Mean total water intake (g)		Mean daily water intake (mg)		Water intake rate (mg/g/day)	
Hopper (instar)	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
I	15	17.4	42.1	39.2	0.547	0.476	35.8	27.4	849.2	689.9
II	9	9.7	91.5	93.0	0.769	1.029	83.5	106.2	849.2	1141.9
III	9	8.8	203.9	196.1	1.915	1.624	124.7	184.5	611.6	940.0
IV	9	9.0	375.7	389.0	4.218	3.414	444.0	379.3	1181.8	975.1
V	13	11.6	1033.7	801.2	11.197	7.236	835.6	623.7	808.4	778.5
VI	18	15.1	2270.8	1615.0	25.870	17.372	1437.4	1150.5	632.9	712.4
Adult (fortnight)										
I	15	15	4671.9	2410.4	41.221	15.748	2748.0	1049.9	588.2	435.6
II	15	15	6087.5	2713.7	21.593	7.322	1439.0	488.1	236.5	179.9
III	15	15	6525.0	2713.7	29.815	9.963	1987.6	664.2	304.6	244.8
IV	15	15	5881.4	2824.9	22.570	9.238	1504.6	615.8	255.8	217.9
V	15	15	5973.4	2889.6	11.119	4.689	741.3	312.5	124.1	108.1

Table 4. Dietary water loss of female *P. pictus* fed *ad libitum* on *C. gigantea* at $26 \pm 2^\circ\text{C}$, $70 \pm 20\%$ r.h. and 8 hr/day photoperiod.

Life stage	Average duration (day)	Mean total water loss via faeces (g)	Rate of water loss via faeces (mg/g/day)	Mean total water loss via transpiration (mg)	Rate of water loss via transpiration (mg/g/day)
Hopper (Instar)					
I	30	0.255	238.2	86.5	80.9
II	16	0.433	264.6	411.0	251.8
III	19	0.867	220.3	661.9	169.0
IV	21	1.961	237.4	926.8	112.2
V	27	5.318	215.8	2794.6	89.1
VI	49	11.445	116.2	4949.3	50.2
Adult	105	33.874	639.4	13835.3	26.3

faeces and transpiration as function of life stages, sex and temperature are reported in tables 4, 5, 6 and 7. Water loss *via* faeces and transpiration at two temperature levels and water loss owing to egg laying were added up to present the total value for female adult. This was necessary as different test individuals oviposited during different fortnights.

Total water loss *via* faeces increased from about 0.25 g in first instar male and female at 26°C to a maximum of 11.44 and 9.89 g in the sixth instar female and male (tables 4 and 5). At 36°C maximum total water loss occurred during the sixth instar, the values being 10 g for male and 13.43 for female. From hatching to death, a female or male at 26°C lost 54.15 or 37.48 g dietary water *via* faeces, whereas the values at 36°C were 96.16 g or 36.26 g. It is evident that as much as 30.69 g or 13.87 g and 74.68 g or 41.85 g of dietary water was absorbed from hatching to death by a female

or male at 26 and 36°C respectively. In terms of assimilation efficiency (Delvi and Pandian 1971), water absorption efficiency was 36.1 or 27.0% at 26°C and 43.7 or 53.8% at 36°C for a female or male respectively. These values may be compared with the assimilation efficiency values reported by Delvi (1972) for *P. pictus* (26°C: male: 27% female: 33%; 36°C: male 56%, female: 58%). It can be seen that a close relationship exists between the efficiencies of matter assimilated and water absorbed.

Table 5. Dietary water loss of male *P. pictus* fed *ad libitum* on *C. gigantea* at 26 ±2°C, 70 ±20% r.h. and 8 hr/day photoperiod.

Life stage	Average duration (day)	Mean total water loss via faeces (g)	Rate of water loss via faeces (mg/g/day)	Mean total water loss via transpiration (mg)	Rate of water loss via transpiration (mg/g/day)
Hopper (Instar)					
I	31	0.243	234.2	82.4	79.8
II	15	0.554	457.8	339.6	280.8
III	16	0.731	251.2	539.6	185.5
IV	24	1.588	175.9	647.4	71.7
V	32	4.774	187.1	1767.6	69.3
VI	40	9.891	166.6	4346.8	73.2
Adult (fortnight)					
I	15	4.068	136.7	943.2	31.7
II	15	4.182	124.5	772.3	22.9
III	15	3.123	91.7	433.1	12.7
IV	15	2.579	65.8	529.0	13.5
V	15	2.133	54.1	481.1	12.2
VI	15	1.806	49.4	487.0	13.3
VII	15	1.806	56.1	487.0	15.1

Table 6. Dietary water loss of female *P. pictus* fed *ad libitum* on *C. gigantea* at 36 ±2°C, 60 ±10% r.h. and 12 hr/day photoperiod.

Life stage	Average duration (day)	Mean total water loss via faeces (g)	Rate of water loss via faeces (mg/g/day)	Mean total water loss via transpiration (mg)	Rate of water loss via transpiration (mg/g/day)
Hopper (Instar)					
I	15	0.307	486.4	215.8	341.7
II	9	0.391	474.3	329.2	399.7
III	9	0.840	457.7	951.7	518.6
IV	9	0.223	814.9	3743.6	1106.8
V	13	5.534	411.8	5053.8	376.1
VI	18	13.429	328.5	11268.8	275.6
Adult	75	75.433	945.0	42049.0	105.3

Table 7. Dietary water loss of male *P. pictus* fed *ad libitum* on *C. gigantea* at $36 \pm 2^\circ\text{C}$, $60 \pm 10\%$ r.h. and 12 hr/day photoperiod.

Life stage	Average duration (day)	Mean total water loss via faeces (g)	Rate of water loss via faeces (mg/g/day)	Mean total water loss via transpiration (mg)	Rate of water loss via transpiration (mg/g/day)
Hopper (Instar)					
I	17.4	0.321	337.2	221.1	324.2
II	9.7	0.458	507.6	519.6	575.9
III	8.8	0.746	431.9	768.9	445.5
IV	9.0	1.617	461.6	1616.0	461.6
V	11.6	3.615	389.0	3143.7	338.2
VI	15.1	10.001	410.1	6618.9	273.1
Adult (fortnight)					
I	15	1.623	44.9	13729.2	235.0
II	15	3.276	80.4	3954.2	97.1
III	15	6.001	147.4	3962.3	97.3
IV	15	5.003	118.8	4065.1	95.9
V	15	3.675	86.4	306.1	7.0

Table 8. Water balance of *P. pictus* and *D. chrysippus* fed *ad libitum* on *C. gigantea*.

Parameter	<i>P. pictus</i>				<i>D. chrysippus</i>	
	26°C		36°C		32°C	
	Female	Male	Female	Male	Female	Male
Intake	84.847	51.351	170.838	78.111	3.689	3.511
Absorption	30.694	13.873	74.681	41.855	1.916	2.000
Loss via						
(a) Faeces	54.153	37.478	96.157	36.256	1.773	1.511
(b) exuvia	0.026	0.026	0.026	0.026	—	—
(c) reproduction	4.548	—	5.519	—	—	—
(d) transpiration	23.168	12.597	64.615	39.614	1.186	0.865
(e) at death pupation	2.952	1.250	4.521	2.215	0.730	1.135
					Mean of ♀ and ♂	
Intake rate	270.4	284.0	734.6	473.4	1453.4	
Absorption rate	97.6	76.7	321.0	254.7	790.7	
Transpiration rate	73.6	69.7	277.7	241.0	415.9	
Efficiency of water absorption (%)	36.1	27.0	43.7	53.8	51.9	56.9

The value for items 1 to 3 are given in g, and those from 4 to 6 in g/g live insect/day.

Rates of dietary water loss *via* defecation or transpiration (Koidsumi 1935; Uvarov 1948) followed the same trend as that of rates of dietary water intake. The rate of water loss *via* faeces was maximum during the second instar at 26°C in female (264.6 mg/g/day) or male (457.8 mg/g/day) and at 36°C in male (507.6 mg/g/day). The transpiration rate in female or male at 26°C was 251.8 or 280.8 mg/g/day (second instar) and in male at 36°C was 575.9 mg/g/day. Rates of water loss *via* faeces or transpiration in females at 36°C was maximum during fourth instar (*via* faeces 814.9; *via* transpiration: 1106.8 mg/g/day). From these respective maxima, the rates of water loss *via* transpiration and defecation decreased as a function of life stage. On the whole, water lost through transpiration in a female or male was 23.17 g or 12.597 g at 26°C, whereas it was as much as 64.615 g in a female or 39.614 g in a male at 36°C (table 8).

Jakovlev and Kruger (1953) reported transpiration rate for a number of grasshoppers. The recalculated values (expressed in mg/g/day) from the basic data reported by them are: 374.4 and 175.7 for male and female of *Chorthippus apricarius* respectively; 326.9 or 191.5 for male or female of *C. parallelus*; 207.4 or 102.2 for male or female of *C. brunneus*; 265 or 132.5 for male or female of *Omocestus viridulus*; 185.8 or 119.5 for male or female of *Mecostethus grossus*; 169.9 or 102.2 for male or female of *Oedipoda caerulescens*. It can be seen from tables 4 to 7 that the transpiration rates reported for the adult *P. pictus* are low, when compared to those of Jakovlev and Kruger (1953). The values reported for *P. pictus* represent only a fraction of the total transpiration, *i.e.* only the dietary water transpired and not total transpiration as in the case of Jakovlev and Kruger (1953). Secondly, the life stage of the grasshoppers studied by them has not been reported; perhaps, they have used different life stages of different species, as their values reported range from 102.2 to 374.4 mg/g/day.

The mean dietary transpiration rate for the entire life span (average transpiration rate, from hatching to death is calculated as the mean transpiration rates of all instars and adult stages of the animal giving due consideration for the duration of each life stage) amounted to 73.6 or 69.7 mg/g/day for female or male *P. pictus* at 26°C and 277.7 or 241 mg/g/day for female or male at 36°C. The mean dietary water transpiration of *D. chrysippus* (from hatching to pupation) at 32°C was recalculated to be 415.9 mg/g/day. In *D. chrysippus* (final instar only) the transpiration rate of water increased from 208 mg/g live insect/day at 20°C to 337, 420 and 1127 at 25, 30 and 35°C, respectively (Pandian *et al* 1978). It is evident that the transpiration rates of *P. pictus* at 36°C or *D. chrysippus* at 32°C are nearly 4 times faster than those obtained for *P. pictus* at 26°C. The transpiration rate of water seems to increase with the increase in temperature.

Total water loss *via* exuvia increased from 0.2 mg in the first instar to 16.6 mg in the sixth instar in either sex and the tested temperature levels. The total water lost *via* exuvia was 2.6 mg. A female or male *P. pictus* at death contained 2.95 or 1.25 g at 26°C and 4.52 or 2.22 g at 36°C. *P. pictus* oviposited minimum twice at 26°C or 3 times at 36°C; the number of eggs oviposited ranged from 127 to 457, along with the oviposited eggs a froth like substance was also deposited. Since oviposition last for over 1 to 3 hr, the flowing froth-like substance began to evaporate, therefore water lost *via* oviposition was estimated by determining the water content of sampling individuals reared at 26 and 36°C before and after ovipositions. Water loss owing to oviposition in a female averaged 4.548 g at 26°C and 5.519 g at 36°C.

Dietary water budget of the tropical orthopteran *Poecillocerus pictus* as functions

of sex and temperature is presented in table 8. For comparison, dietary water budget of a tropical lepidopteran *Danaus chrysippus* obtained by recalculating the basic data of Delvi (unpublished) is also given in table 8. The water loss owing to the transfer of sperm in male *P. pictus* has not been estimated, but it should be negligible. Hence water loss *via* transpiration in male individuals at both temperature levels included a small fraction of water loss due to the sperm transfer. The mean dietary water intake and absorption rate for the entire life span amounted to 270.4 or 97.6 mg/g/day for a female and 284 or 76.7 mg/g/day for male at 26°C respectively. A female or male absorbed 36 or 27% of the total dietary water, about 75.4 or 90.8% of the absorbed water was transpired and 0.09 or 0.19% was lost *via* exuvia (table 8) and 9.6 or 9% at death. A female *P. pictus* lost only 14.8% of water *via* oviposition.

A more or less similar pattern of dietary water distribution is seen in *P. pictus* reared at 36°C. The major differences between individuals reared at two temperatures may be summarised as follows: (i) The rate of dietary water intake or absorption at 26°C did not differ much as a function of sex and averaged 277.3 or 86.6 mg/g/day; at 36°C these values were 734.6 or 321 and 473.4 or 254.7 mg/g/day for a female or a male respectively. (ii) Mean water absorption efficiency varied little with sex and was about 31% at 26°C and 48.7% at 36°C; the water absorption efficiency decreased as the life stages advanced at 26°C; the efficiency values did not significantly vary among individuals belonging to different life stages at 36°C. (iii) A female lost as much as 14.8% water *via* oviposition at 26°C, whereas it was only 7.4% in individuals reared at 36°C. (iv) A female or a male transpired 75.4 or 90.8% at 26°C while the values were 86.5 or 94.6% at 36°C. (v) Male and female individuals carried about 9% of the total dietary water absorbed at death at 26°C, those at 36°C carried only about 6 and 7%. Owing to oviposition a female at 26°C lost 14.8% of the total absorbed water while at 36°C it lost 7.4%.

From the point of view of transpiration rate, water budget of *D. chrysippus* at 32°C is more comparable to that of *P. pictus* at 36°C. Average dietary water intake or absorption rate for *P. pictus* at 36°C, from hatching to death, amounted to 734.6 or 321, 473.4 or 254.7 mg/g/day for female or male respectively. The corresponding value for *D. chrysippus* (dietary water intake rate 1453.4 mg/g/day and absorption rate 790.7 mg/g/day) is approximately 2 to 3 times faster. However, the efficiencies with which water was absorbed in *P. pictus* at 36°C (48.7%) and *D. chrysippus* (54.4%) were more or less similar. The fraction of absorbed water carried by *D. chrysippus* (49.2%) prior to pupation, though not strictly comparable to *P. pictus* at death (about 6% at 36°C), was far high; at 36°C male and female *P. pictus* belonging to sixth instar contained 13 and 9.3% of the respective absorbed water before undergoing final moult. The high fractions of absorbed water contained by *D. chrysippus* before pupation may be an adaption to tide over the non-feeding pupal stage (Hackman 1971).

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Effects of different ration levels on survival, moulting and food conversion in two freshwater prawns

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Abstract. Effects of different daily rations of the oligochaete worm *Tubifex tubifex* on survival, moulting and food conversion of *Macrobrachium lanchesteri* (Palaemonidae) and *Caridina weberi* (Atyidae) have been described. Moulting is a metabolic necessity for either species and occurs even at the expense of organic reserves of starving prawns. The geometric derivation of the growth-feeding rate relations in *M. lanchesteri* indicated that, 88, 162 and 204 mg live food/g live prawn/day represent the maintenance, optimum and maximum feeding rates respectively. The corresponding values for *C. weberi* were 96, 236 and 396 mg. Food availability has a marked influence on the food partitioning and biochemical composition of either species.

Keywords. Ration levels; survival; moulting; conversion efficiency; *Macrobrachium lanchesteri*; *Caridina weberi*.

1. Introduction

Decapod crustaceans of the sub-order Natantia (prawns and shrimps) form a major aquatic resource for human utilization. During the last decade considerable interest has been evinced in India for developing prawn fisheries (Verghese 1978; Jhingran 1982). For successful culture of any species of prawns, the size of the daily meal to be offered is to be established. While the influence of ration levels (size of meal) on the bioenergetics of feeding and growth of fishes have been widely studied (Brett *et al* 1969; Gerking 1971; Brett and Shelbourn 1975; Reddy and Katre 1979), there is a need to extend similar studies in decapod crustaceans (see Katre and Reddy 1976; Sedgwick 1979). In this paper, effects of different ration levels on the survival, moulting, food intake, conversion and yield of two Natantians, *Macrobrachium lanchesteri* and *Caridina weberi* are described.

2. Material and methods

2.1 Maintenance and feeding of prawns

M. lanchesteri (de Man) and *C. weberi* (de Man) were collected from freshwater habitats of Bangalore (77°33 E-12°55 N) and maintained in separate stocking tanks containing 20 l freshwater and fed on an *ad libitum* food of oligochaete worms. After acclimatization to the laboratory conditions for a week, the prawns were starved for 3 days to ensure evacuation of their guts. Active intermoult individuals identified by

the method of Peebles (1977), were selected from this stock and maintained in groups of 4 (*M. lanchesteri*: live body weight 408 ± 35 mg) or 8 (*C. weberi*: live body weight 123 ± 7 mg) in separate aquaria (size: $38 \times 38 \times 25$ cm) containing 15 l freshwater. Throughout the experimental period, a daily photoperiod cycle of 10L:14D (L=0800-1800 hr. D=1800-0800 hr) was maintained and the water temperature was $23.2 \pm 1.6^\circ\text{C}$.

Tubifex tubifex (Muller) which are known to constitute a rich source of nutrition for freshwater prawns (Subrahmanyam 1980; Ponnuchamy *et al* 1981a), were used as food in the present experiment. The different ration levels offered, calculated as percentages of the initial live body weight of the prawns were 0, 1, 3, 6, 12, 15, 20 and 25 for *M. lanchesteri* and 0, 3, 6, 12, 24, 30, 35 and 40 for *C. weberi*. Four replicates were maintained for each feeding level. Feeding and collection of uningested food was undertaken daily between 1000 hr and 1200 hr. During the 60-day experimental period, water was aerated continuously. Since no qualitative analyses of the faecal matter was undertaken, fortnightly collection of faecal matter by filtration provided adequate quantitative data on the production of faeces by the experimental prawns.

2.2 Moulting record

Daily, while removing the uningested food, the moults produced by the experimental prawns were collected. After blotting the adhering water, the moults were weighed and dried overnight in a hot air oven (90°C) to record the dry weight. Moulting frequency, total moulting weight and mean moulting weight of the prawns were calculated as follows:

$$\text{Average moulting frequency (days)} = \frac{\text{Experimental duration} \times \text{Number of prawns}}{\text{Number of moults produced}}$$

Total moulting weight = Pooled dry weight (mg) of all the exuviae produced per prawn during the entire experimental period.

Mean moulting weight = Moulting dry weight expressed as percentage of the initial dry body weight of the prawn.

2.3 Parameters of feeding and conversion

Food intake, assimilation efficiency and gross and net conversion efficiencies (K_1 and K_2 : %) were calculated following the procedures detailed by Katre and Reddy (1976) and Ponnuchamy *et al* (1981b). The production of new tissues (= conversion) was calculated by adding the exuvial weight to the gain in total weight of the prawn and the actual growth was calculated by subtracting the exuvial weight from the gain in total weight and expressed as "yield". The gross and net yield efficiencies were calculated as follows:

$$\text{Gross yield efficiency (Y}_1\text{, \%)} = \frac{\text{Total weight gain} - \text{exuvial weight}}{\text{Food consumed}} \times 100$$

$$\text{Net yield efficiency (Y}_2\text{, \%)} = \frac{\text{Total weight gain} - \text{exuvial weight}}{\text{Food assimilated}} \times 100$$

Since the duration of the experiment was fairly long, the feeding and conversion rates are calculated on the basis of mean body weights (=mean of initial and final body weight) of the animals and represented as mg dry/g live prawn/day. The data was statistically compared by the students 't' test (Fisher 1950).

2.4 Biochemical parameters

Biochemical constituents of initial and experimental prawns were estimated in dried samples. The water content was calculated as the difference between live and dry weights of the material. Carbohydrate was determined by estimating the glycogen content following the phenol-sulphuric acid method (Dubois *et al* 1956). Fat was estimated by extracting a known amount of the material (35 to 45 mg) in 2:1 chloroform-methanol mixture, using a Soxhlet apparatus. Each extraction was run for 20 hr, as suggested by the Association of Official Agricultural Chemists (1950). Ash was estimated by incinerating a known amount of material (35 to 45 mg) in a Muffle furnace at 560°C for 5 hr (Paine 1964). The percent protein value including undetermined chitin content was derived by subtracting the determined percent values of fat, carbohydrate and ash from 100 and expressed as "crude protein".

3. Results and discussion

3.1 Survival

M. lanchesteri and *C. weberi* showed 100% survival at 6% ration level and beyond. While total mortality of starved *M. lanchesteri* occurred within 53 days, starved *C. weberi* indicated only 15.6% mortality during the experimental period. A lower survival (18.7%) was observed for *M. lanchesteri* receiving 1% ration level, as compared to 3% ration level (85%). However, *C. weberi* receiving 3% ration level showed a higher survival (93.7%).

3.2 Moulting production and moulting frequency

At lower feeding levels, the moulting frequency of the prawns was low. Reeve (1969) also reported that in *Palaemon serratus* lower feeding levels interfere seriously with the fine control mechanism of moulting, producing widely different intermoult periods. However, as compared to that of *P. lamarrei* (Katre and Reddy 1976), the intermoult durations of *M. lanchesteri* observed presently are higher. While there were no marked differences in the mean moult weight of *M. lanchesteri* in relation to ration level (table 1), Katre and Reddy (1976) have cited a decrease in the per moult weight with increase in ration levels of *P. lamarrei*. Consequent to frequent moulting with increase in ration level, the total moult weight of *M. lanchesteri* increased markedly. Observations on the intermoult durations and total moult weights of *C. weberi* in relation to ration level indicated similar changes as observed for *M. lanchesteri*. However, the mean moult weight of *C. weberi* increased with increases in ration level.

Starvation and lower ration levels seem to retard moulting to a certain extent in both the species of prawns. This is in conformity with the observations of Katre and Reddy (1976) for *P. lamarrei* and other crustaceans (Pyle 1943; Costlow and Bookhout 1953, 1957). Even starved individuals of *P. lamarrei* were known to exhibit regular moulting, though they were shown to decrease in their final body weights (Katre and Reddy 1976). Interestingly, Lasker (1966) reported that, despite the presence of food, the shrimp *Euphausia pacifica* did not exhibit growth but underwent moulting with a final decrease in the body weight. This indicates that moulting is a metabolic necessity in these natantians and that it continues even at the expense of the organic

Table 1. Influence of ration levels on the moult production and moulting frequency of the two natantians.

Ration level (%)	Moult produced (number)	Moulting frequency (days)	Total moult weight (mg)	Mean moult weight (%)
<i>Macrobrachium lanchesteri</i>				
0	0.25±0.00	—	2.31±0.00	10.32±0.46
1	0.62±0.12	—	7.68±0.00	12.59±1.25
3	0.62±0.12	—	4.52±1.89	8.90±1.23
6	0.83±0.24	—	6.30±3.28	9.89±1.43
12	1.75±0.25	34.76±4.09	11.43±1.43	8.60±1.36
15	1.67±0.24	35.50±6.35	12.99±4.71	9.63±1.04
20	1.94±0.26	30.92±3.76	17.81±4.36	9.67±1.46
25	1.88±0.38	27.90±6.10	13.06±3.80	10.37±1.76
<i>Caridina weberi</i>				
0	0.97±0.09	—	2.02±0.78	8.78±0.69
3	1.00±0.01	—	2.99±0.65	10.10±0.94
6	2.62±0.35	23.27±3.21	8.76±2.17	11.23±1.17
12	3.17±0.54	18.75±2.76	10.40±1.66	12.07±0.51
24	3.80±1.02	16.00±2.89	14.24±2.67	13.00±1.26
30	3.90±0.64	15.77±2.77	17.08±3.02	14.78±1.06
35	4.05±0.20	14.60±0.70	15.92±1.57	14.29±1.24
40	4.35±0.60	13.90±1.21	17.29±1.02	14.57±0.68

reserves of the animal. In contrast to this, Roberts (1957) reported that, starvation inhibited moulting in the crab *Pachygrapsus grassipes*.

3.3 Food offered and food ingested

Table 2 indicates different ration levels offered, the food ingested by and the growth of *M. lanchesteri*. The food offered was consumed up to a ration level of 6%. Beyond this level, food ingested was less than the food offered. However, with increase in the food offered beyond 6%, there were increases in the food ingested reaching a maximum of 18% at the highest ration level (25%).

Table 3 indicates the food offered, food ingested by and the growth of *C. weberi*. Food was fully ingested upto a ration level of 12%, beyond which the food ingested was lower than that offered. However, as in *M. lanchesteri*, with further increases in ration level offered, there were increases in the food ingested reaching a maximum of 32.5% of the animal's initial body weight.

The differences in feeding rates of *M. lanchesteri* at 15% ration level and above were significant ($P > 0.05$) indicating that, 15% is the maximum that this prawn can ingest under the present experimental conditions. In *C. weberi* the differences in feeding rates beyond 32% ration level were insignificant ($P > 0.9$). This indicates that *C. weberi* accepts a maximum of 32% of food under the experimental conditions which is nearly twice that accepted by *M. lanchesteri*. Smaller body size and correlated higher metabolic rate may be the factors governing the higher rates of feeding in *C. weberi*.

Table 2. Influence of ration levels on the body weight, food intake and growth of *Macrobrachium lanchesteri*.

Ration level (%)	Initial dry body weight (mg)	Final dry body weight (mg)	Food offered (mg dry food/g live prawn/day)	Growth (mg dry weight/prawn/day)
0	93.46±9.19	34.71±6.80	—	-1.70±0.35
1	91.11±5.61	39.98±9.35	1.93±0.12	1.40±0.17
3	94.87±2.63	63.46±7.17	5.28±0.17	0.55±0.11
6	93.22±8.50	80.21±8.04	10.34±0.20	0.22±0.07
12	83.75±4.60	103.92±9.82	19.80±0.73	+0.34±0.14
(10.8)			(17.91±0.86)	
15	94.96±6.38	139.30±8.87	25.32±1.33	0.74±0.11
(12.5)			(21.18±2.81)	
20	92.10±7.15	116.68±5.57	27.27±0.27	0.41±0.17
(13.8)			(18.88±2.66)	
25	84.63±6.96	101.94±9.79	34.46±0.35	0.30±0.20
(18)			(24.76±3.56)	

The numbers in the parentheses represent the amounts of food ingested by the prawn at each ration level.

Table 3. Influence of ration levels on the body weight, food intake and growth of *Caridina weberi*.

Ration level (%)	Initial dry body weight (mg)	Final dry body weight (mg)	Food offered (mg dry food/g live prawn/day)	Growth (mg dry weight/prawn/day)
0	27.47±1.00	10.43±0.53	—	-0.30±0.02
3	29.27±1.29	19.04±0.91	5.21±0.12	0.17±0.03
6	29.24±1.58	27.49±1.75	10.20±0.13	0.03±0.01
12	27.11±1.29	29.96±4.71	20.53±0.17	+0.05±0.02
24	28.71±1.57	35.25±1.09	41.14±3.51	0.11±0.03
(21.2)			(36.29±3.52)	
30	29.56±2.25	41.52±3.53	49.23±0.14	0.20±0.09
(24.5)			(40.23±1.74)	
35	27.67±1.44	42.82±2.95	55.93±1.17	0.25±0.03
(32.5)			(51.98±2.76)	
40	27.41±0.60	42.86±1.88	64.01±0.30	0.26±0.04
(32.4)			(51.95±4.54)	

The numbers in the parentheses represent the amounts of food ingested by the prawn at each ration level.

3.4 Metabolism and growth

A daily ration level of 6% body weight was insufficient for the growth of *M. lanchesteri* (table 2). At higher feeding levels, considerable growth occurred, the maximum being 0.74 ± 0.11 mg at 15% ration level. This ration level is interestingly equivalent to the *ad libitum* ration level determined for these prawns held at the ideal population

density levels (Ponnuchamy 1981). In *P. lamarrei*, maximum growth was recorded at a ration level of 10% (Katre and Reddy 1976). At higher ration level, inspite of higher food consumption (table 2) the growth of *M. lanchesteri* decreased, perhaps due to decreased assimilability of the prawns (see Monokov 1972).

A daily ration level of 6% was also insufficient for the growth of *C. weberi* (table 3). At higher feeding levels, considerable growth ensured, reaching a maximum of 0.26 ± 0.04 mg at the highest ration level offered (40%). However, increases in growth almost approached an asymptote, beyond a ration level of 30%. In spite of differences in their optimum ration levels, both the species indicated growth only beyond the ration level of 12%. This indicates that both the species expend considerable energy for their maintenance.

Physiologically useful energy in excess of metabolic requirements of animals, is available for deposition as body tissue during growth, fat depot and/or for synthesis during the reproductive process. The geometric derivations (*cf* Thomson 1941) of the growth-feeding rate relations for *M. lanchesteri* indicated that 88, 162 and 204 mg food/g live prawn/day represent the maintenance, optimum and maximum feeding rates of the prawn respectively (figure 1). While these values are higher than those reported for *P. lamarrei* (Katre and Reddy 1976), the maximum feeding rate of *M. lanchesteri* (126.7 mg) is lower than that of *P. lamarrei* (179.5 mg). The differences may be due to the differences in the size of the two species of prawns.

Figure 2 represents the quantitative relations of the feeding rates to the food absorbed, metabolised for body functions and converted into new body substance in *M. lanchesteri*. From the estimated maintenance ration (figure 1), the level of specific dynamic action (SDA : of Brody 1968) or calorigenic effect (Kleiber 1961) could be derived. SDA represents the energy requirements of the animal for digestion, assimilation, transportation, biochemical treatment and incorporation (Beamish 1974). An increase in the SDA from 2.5 mg at the optimum level to 6.5 mg at the maximum

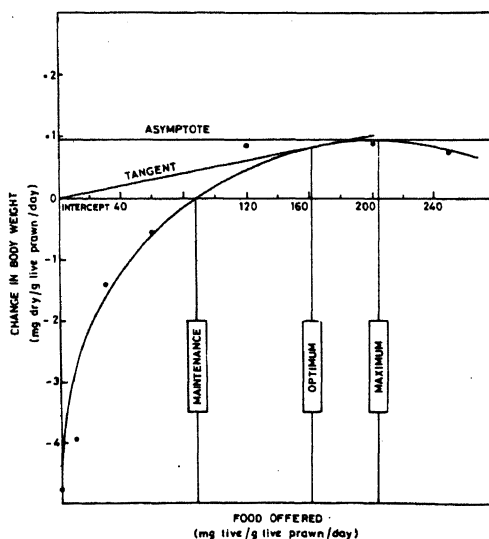


Figure 1. Geometric derivations of maintenance, optimum and maximum feeding rates for *Macrobrachium lanchesteri*.

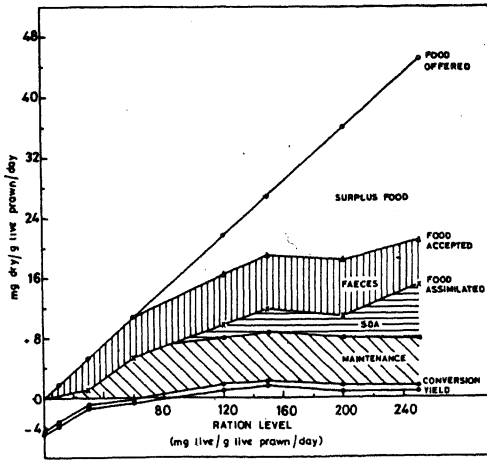


Figure 2. Quantitative partitioning of food in *Macrobrachium lanchesteri*.

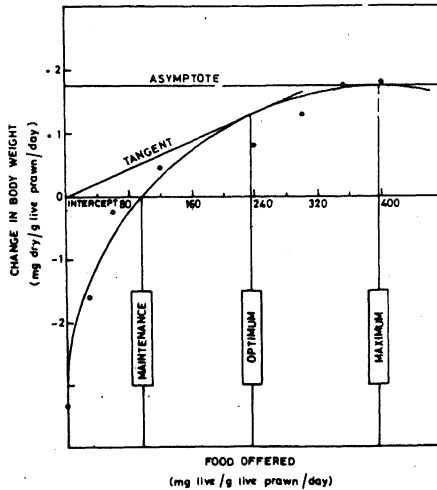


Figure 3. Geometric derivations of maintenance, optimum and maximum feeding rates for *Caridina weberi*.

ration level is evident. Higher SDA for individuals fed near maximum rations have also been reported for *P. lamarrei* (Katre and Reddy 1976).

From the geometric derivations of the growth-feeding rate relations for *C. weberi* (figure 3), it is evident that 96, 236 and 396 mg/g live prawn/day represent the maintenance, optimum and maximum feeding rates respectively. The observed value of 282.3 mg is rather low as compared to the geometrically-derived maximum.

The quantitative relationships between the feeding rates and energy budget of *C. weberi* are represented in figure 4. The SDA increased from 11 mg at optimum ration level to 17 mg at the maximum ration level indicating the considerable energy cost of converting food into body substance at the higher ration levels. Higher values of maintenance, optimum and maximum feeding rates were observed in *C. weberi* as compared to *M. lanchesteri*. Comparatively higher SDA was also observed for *C. weberi* than for *M. lanchesteri*.

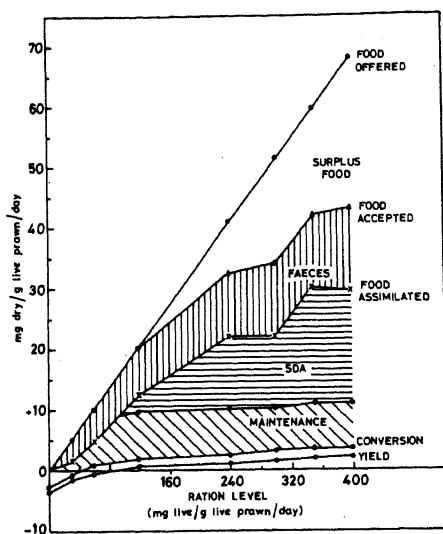


Figure 4. Quantitative partitioning of food in *Caridina weberi*.

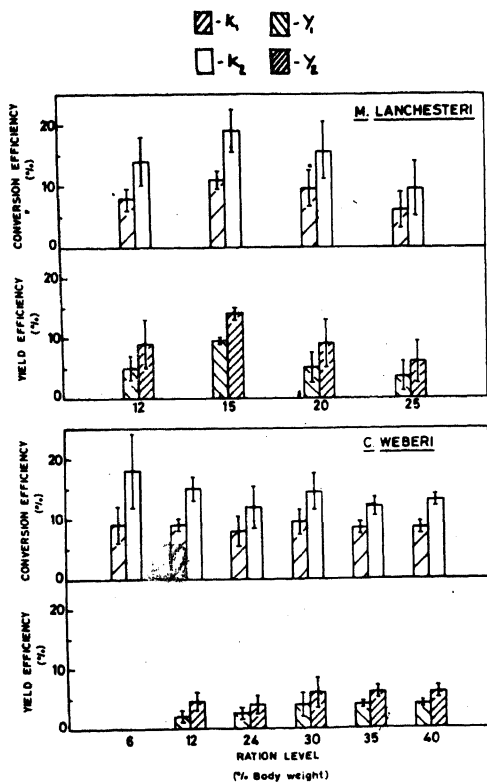


Figure 5. Influence of ration levels on the gross and net conversion/yield efficiencies of the two natantians.

3.5 Conversion and yield efficiencies

Figure 5 represents the conversion efficiency and yield efficiency of the two species in relation to different ration levels. Maximum net yield efficiency was observed in *M. lanchesteri* at the ration level of 15% which is equivalent to the maximum ration level accepted. Katre and Reddy (1976) observed a peak conversion efficiency for *P. lamarrei* at the ration level of 10%. However, high net yield efficiency was observed at the higher ration level (30% and above) for *C. weberi*.

Reviewing the studies on feeding in invertebrates, Monokov (1972) reported that under experimental conditions with increase in ration levels and extra feeding, there may be decreases in the assimilability of food which alters the net yield efficiency. In *Penaeus japonicus*, Shigeno *et al* (1972) reported that a high rate of food intake does not always bring out an effective growth rate, thereby leading to lower conversion efficiency. During the present study, while the above was true for *M. lanchesteri*, *C. weberi* indicated high values of yield efficiency at the higher ration levels. Further, increases in the ration size beyond the highest ration level perhaps results in decreases in the yield efficiency of *C. weberi*.

3.6 Biochemical composition

The changes in the biochemical contents of control and experimental *M. lanchesteri* are indicated in table 4. The water content was maximum in starved individuals (88.5%) and is comparable to that reported for *P. lamarrei* (89.1%; Katre and Reddy 1976). Starvation is known to increase the water content of fish also (Phillips *et al* 1960; Pandian and Raghuraman 1972). While the content of ash decreased with increase in the ration level, those of fat, carbohydrate and crude protein (including chitin) did not show significant variation till 6% ration level. However, the biochemical composition of control and experimental prawns receiving a ration level of 12% and above, indicated almost similar values. The differences in all the biochemical constituents were distinct between the prawns receiving the lower (below 6%) and higher levels (above 12%) of ration.

Table 4. Changes in the biochemical composition of *Macrobrachium lanchesteri*.

Ration level (%)	Water content	Dry matter	Ash	Fat	Carbohydrate	Crude protein including chitin
Control	77.67±2.39	22.33±2.39	13.28±0.42	31.22±1.63	1.56±0.14	53.94
0	88.54±0.66	11.46±0.66	20.94±0.30	19.67±4.25	1.37±0.12	58.02
1	86.92±0.58	13.08±0.58	20.33±0.69	19.39±2.14	1.59±0.27	58.69
3	81.32±2.46	18.68±2.46	15.79±0.07	21.31±1.76	1.81±0.17	61.09
6	79.48±0.52	20.52±0.52	15.31±0.42	21.57±0.90	1.86±0.21	61.26
12	75.08±1.53	24.92±1.53	13.05±1.16	26.72±1.95	1.42±0.18	58.81
15	71.20±1.49	28.80±1.49	13.73±0.54	28.59±3.24	1.36±0.22	56.32
20	75.28±1.76	24.72±1.76	14.07±0.35	34.40±2.16	1.41±0.22	50.12
25	76.65±1.49	23.35±1.49	13.45±0.46	30.07±3.80	1.45±0.06	55.03

While water and dry matter are represented as percent live matter, others are represented as percent dry matter.

Table 5. Changes in the biochemical composition of *Caridina weberi*.

Ration level (%)	Water content	Dry matter	Ash	Fat	Carbohydrate	Crude protein including chitin
Control	77.28±1.67	22.72±1.67	13.53±0.18	31.22±1.63	1.52±0.23	53.73
0	82.05±1.33	17.95±1.33	16.69±0.19	15.79±2.27	1.56±0.09	65.96
3	78.15±0.81	21.85±0.81	14.52±0.80	19.54±1.32	1.51±0.14	64.43
6	75.09±0.45	24.91±0.45	14.35±0.63	19.05±4.43	1.73±0.21	64.87
12	74.45±1.03	25.55±1.03	13.77±0.24	24.85±1.39	1.68±0.22	59.70
24	76.79±0.49	23.29±0.49	12.48±0.62	38.79±1.21	1.68±0.24	45.05
30	76.16±1.15	23.84±1.15	13.10±0.13	39.50±0.09	1.64±0.12	45.76
35	74.57±0.57	25.43±0.57	13.06±0.70	33.97±3.38	2.16±0.36	50.81
40	74.25±0.14	25.75±0.14	13.42±0.90	34.11±4.12	1.76±0.18	50.70

While water and dry matter are represented as percent live matter, others are represented as percent dry matter.

Table 5 indicates the changes in the biochemical constituents of *C. weberi* in relation to different ration levels. As in *M. lanchesteri*, the water content was highest in starved individuals. While the contents of ash decreased with increases in the ration level, that of fat increased. However, the biochemical constituents of prawns receiving higher ration level as well as control prawns showed similar values.

4. Conclusions

From the foregoing account, it is apparent that availability of food has a profound influence on the survival, moulting and food conversion of either species of prawns. While tubificid worms do offer a nutritive source, a poor feed conversion ratio of 34:1 (for *M. lanchesteri*) and 31:1 (for *C. weberi*) at optimal rations, implies that it would be necessary to evolve a nutritionally rich diet, if necessary a compounded one, to obtain better feed conversion ratios.

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Reproductive biology of the larvivorous fish *Macropodus cupanus* (Cuv. and Val.)

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Abstract. Investigations on the maturity stages, pattern of distribution of ova in the ovary, growth of the ova to maturity, minimum size at first maturity, sex ratio, growth rate of the ovary, spawning frequency, spawning season and fish-fecundity relationships of the larvivorous fish *Macropodus cupanus* indicate the extent to which breeding is geared to take maximum advantage of environmental and other factors which afford the greatest opportunity for survival and development of the new generation. The mode of development and growth of the ova reveal that the first maturation is normally delayed till the main period of body growth is over; further, the linear relationships derived between growth rate of the (female) gonads and fish size are a reflection of the symmetry of the gonads. Correlating data on egg size, fecundity and conditions for incubation, *M. cupanus* with its high fecundity and rapid development can be said to incline towards 'r-selection' on the continuum between 'k' and 'r-selection'. Investigations on spawning frequency and season reveal the protracted spawning season from April to September and the shorter one in January and February. The distinct monsoon spawning peak leading to an increase of its population during this season coincides with the peak incidence of mosquito larvae, indicating the larvivorous potential of the fish.

Keywords. Reproductive biology; larvivorous fish; *Macropodus cupanus*.

1. Introduction

Indiscriminate releases of the exotic larvivorous fish *Gambusia affinis* and *Poecilia reticulata* into the aquatic environment for mosquito larval control have threatened the survival of valuable aquatic fauna (Myers 1965; Bay 1973; Menon 1977). This has renewed interest in the biocontrol potential of indigenous larvivorous fish such as *Macropodus cupanus* (Cuv. and Val.). An essential aspect of such investigations is the reproductive cycle and potential. Even though a biocontrol agent may have a high unit preference and feeding capacity for mosquito larvae, its effectiveness in reducing the larval population is a function of its population density (Bay 1972). However, although studies on the eggs and early development of *M. cupanus* have been conducted (Jones 1940; Padmanabhan 1955), detailed information on the gonadal cycle of fecundity and spawning is lacking, which prompted the present study.

2. Material and methods

Regular fortnightly samples of *M. cupanus* collected for one year (March 1977 to February 1978) from Veli lake, Chakai canal and surrounding paddy fields in Trivandrum, were utilised to record data on standard length, weight, stage of sexual maturity and length and weight of the gonad. The criteria used for quantification of maturity were

colour, shape and size of the ovary, diameters of the unspawned eggs and their general appearance, particularly the extent of yolk formation, since male gonads could not be graded macroscopically. To eliminate sampling error resulting from regional differences in the ovary (the anterior region of the ovary of *M. cupanus* has a larger complement of immature ova than the middle and posterior regions, (Jacob 1981), a mixed sample of ova from the different regions was used for all ova diameter studies. Random samples of 1000 ova from ovaries in the different stages of maturity and mature/ripe ovaries were used to elucidate the progression of development of the ova to maturity and types of spawning tendencies following Hickling and Rutenberg (1936) and Prabhu (1956). Specimens were categorized into different length groups on the basis of their stages of maturity, to determine minimum size at first maturity; stages III, IV and V were treated as mature. To delineate the spawning season—through a study of the gonado-somatic index and percentage occurrence of gonads in the different stages of maturity during the different months—females alone were used since male gonads exhibited only microscopically discernible changes, apart from minor alterations in size. Fecundity estimates were based on sub-sampling of unbiased samples of ovaries from gravid fish collected during their peak spawning season (May) as recommended by Bagenal and Braum (1968).

3. Results

3.1 *Delineation of the maturity stages of the female gonads*

In *M. cupanus*, the ovaries have been classified into 5 stages, as suggested by Qasim (1973) for tropical fish.

3.1a *Stage I Immature virgins:* The small, thread-like, whitish, almost transparent ovaries occupy only a minute portion of the abdominal cavity. The ova are invisible to the naked eye, but under the microscope are transparent with a central nucleus. At this stage yolk formation has not yet commenced. The diameter of majority of ova in this stage ranges from 40-60 μ ; the largest measuring 98 μ in diameter.

3.1b *Stage II Maturing virgins/recovered spents:* The semi-transparent, whitish ovaries are larger owing to lateral and antero-posterior expansion. The shape and positioning are distinct; each ovary is triangular when viewed from the side with the apex directed backwards and the anterior extremity of the ovary forming the base of the triangle. The anterior extremities are close together and their ventral angles are united although the posterior two-thirds of each ovary are separated since they extend into the posterior part of the body cavity lying between the haemal processes of the vertebral column. The maturing ova with a mode at 110 μ and a maximum diameter of 165 μ are separated from the general immature stock and are opaque owing to the commencement of yolk formation.

3.1c *Stage III Ripening:* The opaque yellow ovaries are almost fully packed with the fully yolked ripening ova and occupy almost half the total space available posteriorly between the haemal processes of the vertebral column. Anteriorly, however, they extend only slightly into the abdominal cavity, unlike in most other fish. The spherical, fully yolked ova show a mode at 550 μ and a maximum diameter of 716 μ .

3.1d Stage IV Ripe: The massive yellowish-red ovaries with very thin distended ovarian walls, and numerous blood vessels ramifying over the surface, are fully packed with large opaque eggs showing a mode at 750 μ , but with a maximum diameter of 1002 μ . The ovaries now extend deep into the spaces on either side of the vertebral column, almost up to the caudal region posteriorly. The anterior basal portion is deeply concave; the concavities of the two ovaries form a shallow depression into which the intestinal coils fit.

3.1e Stage V Spent: The highly shrunk flaccid blood-shot ovaries bear a superficial resemblance to Stage II, but differ in the loosely-packed nature of the maturing ova, in the scattered transparent areas visible and in the general granular appearance of the ovary. In a recently spawned fish a few remnants of mature ova with a maximum diameter of 680 μ are present as resorbing and disintegrating structures, but the majority of the ova are small, transparent and invisible to the naked eye.

3.2 Development of the ova to maturity

Data from frequency polygons of ova diameter measurements taken from ovaries of different maturity stages (figure 1) reveal the progression of development of ova in *M. cupanus*. In stage I, there are only immature ova, ranging from 23 to 98 μ in diameter with a distinct mode at 50 μ . As maturation progresses, this batch of eggs is separated from the immature stock and represented by a mode at 110 μ in stage II; the maximum ovarian size at this stage is 165 μ . In stage III, this maturing group of ova with a mode at 550 μ shows an increase in size and yolk deposition. A second group of maturing ova with a mode at 350 μ , distinct from the immature stock, is visible at this stage. As development progresses, the second group of maturing ova shows only a slight increase of 100 μ , vide stage IV. The first distinctly separate group

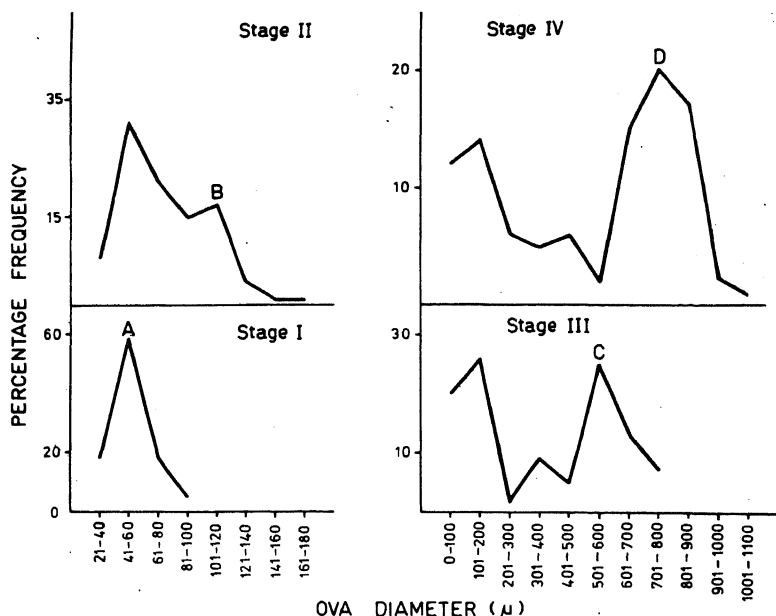


Figure 1. Ova diameter measurements from ovaries of different stages of maturity, showing the progression of development of the ova in *M. cupanus*.

with a mode at 750 μ and a maximum ova diameter of 1002 μ evidently constitutes the mature group of eggs which would be spawned in the ensuing spawning season. It should also be noted that the rate of development of ova is maximum between stages II and III.

3.3 Minimum size at first maturity

The results presented in table 1 show that all specimens below 18 mm are immature; maturity sets in from the 19 mm stage onwards. The length at which 50% of the specimens attain maturity, considered the length at first maturity (Kagwade 1968), lies between 22 and 24 mm.

Table 1. Occurrence of female *M. cupanus* in different stages of maturity in the various size groups.

Standard length (mm)	Immatures (%)	Maturing virgins (%)	Matures (%)
13–15	100.00	—	—
16–18	100.00	—	—
19–21	74.29	20.00	5.71
22–24	51.16	37.21	11.63
25–27	15.22	47.83	36.96
28–30	4.17	16.67	79.17
31–33	3.33	10.00	86.67
34–36	—	7.69	92.31
37–39	—	—	100.00

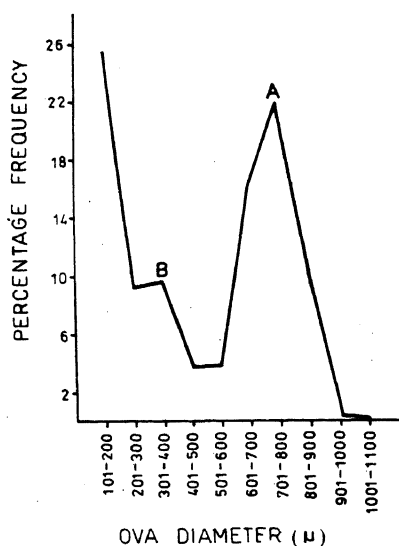


Figure 2. Diameter measurements of 1000 ova from stage IV ovary of *M. cupanus*.

3.4 Spawning frequency

Data (figure 2) indicate that within the ovary, apart from the general immature stock, there are two distinct batches of eggs, represented by the modes *A* and *B*. The group of eggs under mode *A* is in the ripe condition and ready to be spawned, while the second group of eggs at mode *B* is the maturing batch in which yolk formation has already advanced. It is likely to be shed subsequent to the first batch, indicating the possibility of a second spawning. Again, as the time required for the second batch to attain maturity is less than that required for the first batch (de Jong 1939) and as the two batches are not clearly and sharply demarcated, it is likely that this species spawns twice during the spawning season.

3.5 Spawning season

The percentage occurrence of female fish with gonads in various stages of maturity (table 2) reveals that although ripe females are present during all the months except October, November, December and March, a distinct periodicity in breeding is discernible. A major peak in gravid female number is recorded in April/May which then gradually falls, reaching a minimum in August. The spent fish number is high in these months with a maximum in June, after spawning. The number of recovered spents is high in July and August, indicating the conversion of at least a part of the spents into the recovering stage. In September, a second rise in ripe female number is visible. In October and November, after spawning, the number of spents and recovered spents is high; no ripening and ripe fish are obtained during these months.

Table 2. Monthly percentage occurrence of female *M. cupanus* in the different stages of maturity (females 28 mm and above alone are considered).

Months	Stages of maturity				
	I Immature	II Maturing/ recovered spent	III Ripening	IV Ripe	V Spent
March '77	—	80.00	20.00	—	—
April	—	7.14	58.57	34.29	—
May	8.33	—	13.00	51.67	27.00
June	—	—	6.00	30.00	64.00
July	—	17.65	—	29.41	52.94
August	—	9.09	18.18	18.18	54.55
September	—	5.88	5.88	76.47	11.76
October	12.50	57.50	—	—	30.00
November	—	66.67	—	—	33.33
December	—	100.00	—	—	—
January '78	—	38.89	11.11	38.89	11.11
February	—	—	—	30.00	70.00

By December, all specimens collected are in the recovered spent stage. In January, the number of ripe and ripening females begins to increase and another spawning takes place. The number of ripe fish declines in February while the number of spents increases, amounting to 70% of the total collection. In March almost 80% of the population is in the recovered spent stage while a few have gone into the ripening stage. From April the number of ripening and ripe specimens increases until peak spawning in May.

The gonado-somatic indices (figure 3) confirm the pattern described above. There is a long spawning period between April/May and September, with a major peak in May and a smaller one in September. In January the gonado-somatic index peaks again, and then declines till the peak in April/May.

3.6 Sex ratio

The sex composition of random samples examined each month (table 3) shows that the two sex are present in more or less equal numbers since the variation in the number of males and females is negligible during most months.

3.7 Growth rate of the ovaries

The relationships between the parameters of length and weight of the fish and ovary was utilised for following the growth rate of the ovary (table 4). It is evident that the

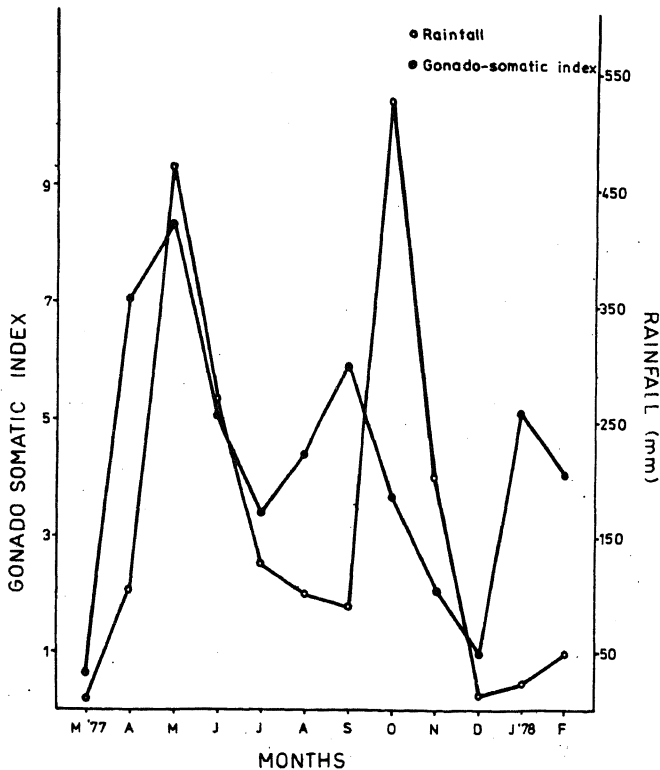


Figure 3. The breeding of *M. cupanus* in relation to rainfall.

Table 3. Sex ratio in *M. cupanus*.

Months	No. of fish examined	Males (%)	Females (%)
March '77	30	46.67	53.35
April	25	36.00	64.00
May	37	48.65	51.35
June	25	44.00	56.00
July	53	45.28	54.72
August	54	50.00	50.00
September	49	38.78	61.22
October	49	48.98	51.02
November	49	55.10	44.90
December	54	55.56	44.44
January '78	58	50.0	50.00
February	65	40.00	60.00

Table 4. Relationship between body growth and ovary growth in *M. Cupanus*.

Variables	Equation	Correlation coefficient
Length of the fish, L Length of the ovary, LO	$LO = 0.4456 L - 3.0080$	0.8504
Weight of the fish, W Weight of the ovary, WO	$WO = 0.1288 W - 23.8846$	0.8699

All correlation coefficients are significant at the 1% level

rate of growth is linear, the ovaries increasing in length and weight in proportion to the increase in length and weight of the fish.

3.8 Fecundity

Figures 4-7 indicate the relationships between fecundity and the variables—length of the fish, weight of the fish, ovary length and ovary weight (a slight asymmetry was noted in certain specimens between the right and left ovaries, but this feature was neither consistent nor significant). The regression equation obtained from relating fecundity and fish length is linear and the correlation coefficient is significant at the 5% level indicating that as fish length increases, fecundity also increases proportionately. However, the relationships between fecundity and fish weight, ovary length and ovary weight are exponential. The coefficient of correlation is significant at the 1% level, indicating that the rate of increase of fecundity, proportionate to these 3 variables is initially fast, but decreases with time.

The relationships between relative fecundity (*i.e.*, the number of eggs per gram body weight of the fish) and the various variables is shown in table 5. *M. cupanus* has a mean relative fecundity of 841. The relationship between this value and standard length and weight is linear, suggesting that relative fecundity increases with increase in these variables. When ovary length and weight are related to relative fecundity, the coefficient of correlation is not significant in both cases, suggesting that these variables do not affect relative fecundity.

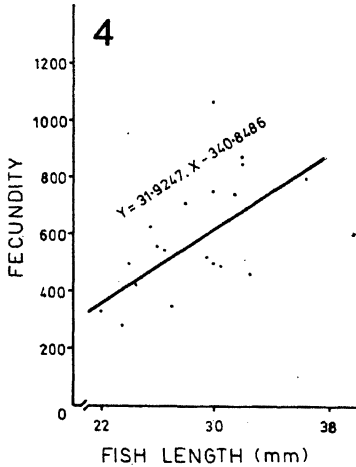


Figure 4. The relationship between fish length and fecundity in *M. cupanus*.

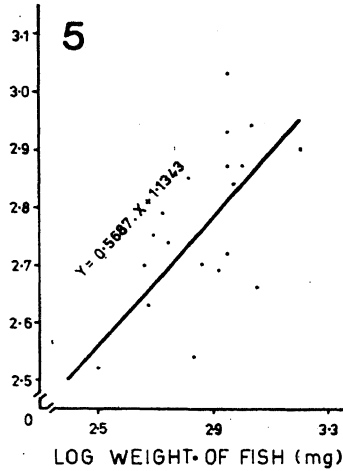


Figure 5. The relationship between fish weight and fecundity in *M. cupanus*.

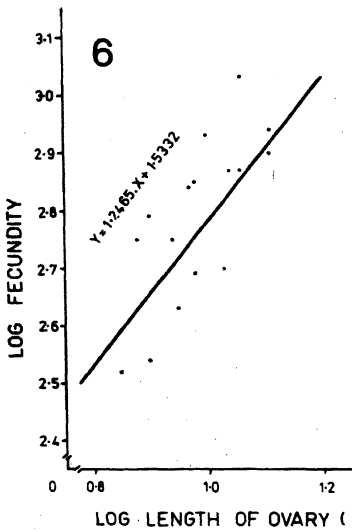


Figure 6. The relationship between ovary length and fecundity in *M. cupanus*.

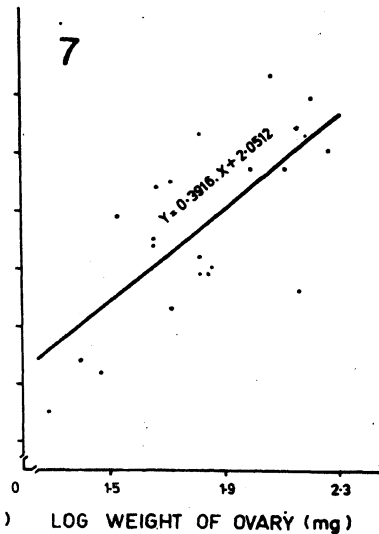


Figure 7. The relationship between ovary weight and fecundity in *M. cupanus*.

Table 5. Relationship between relative fecundity and the different variables in *M. cupanus*.

Variable	Equation	Correlation coefficient
Length of the fish, L	$F = 1.3110 L + 4.8098$	-0.5394*
Weight of the fish, W	$F = 0.4299 W + 4.1304$	-0.5797**
Length of the ovary	Relationships not worked out since correlation coefficients are not	-0.3453
Weight of the ovary	significant	-0.3789

**Significant at the 1% level; *Significant at the 5% level. Other correlation coefficients not significant.

4. Discussion

That a cycle of gonadal maturation, imposed by the energy demands of maturing a batch of eggs/young ones exists, even in regions where environmental conditions are relatively stable and offsprings are produced throughout the year, is undisputed (Peter and Hontela 1978). However, in this study, considering the overall implications of the problems relating to the quantification of maturity, the multi-stage classifications commonly used (Bagenal and Braum 1968) were discarded, and the suggestion of Qasim (1973), that in tropical forms the classification should be limited to 5 maturity stages was followed. Data reveal that as stated by Hoar (1969), gonadal maturity and body growth are linked since the analysis of the body length-gonad length relationships reveals that increase in female gonad length is proportional to fish length; this is partially supported by the work of Rao (1967) in other fish.

Considering the sex ratio, the situation in *M. cupanus* is in contrast to that in other larvivorous fish, specifically *G. affinis* where more females are encountered in the environment (Mallars and Fowler 1970).

Earlier data on the spawning season and frequency are contradictory. Thomas (1870) reported that the fish breeds in May and June but Jones (1940) could collect eggs in January, February, May and from September to November. Padmanabhan (1955) stated that the fish is a perennial spawner with a noticeable increase in the frequency of spawning during the monsoon months. The available data from this investigation indicate that the fish exhibits a protracted spawning from April to September and then a shorter one in January and February. This corresponds to data available for a large number of West coast species (Qasim 1973). It must be mentioned that females alone were considered for the demarcation of the spawning season since male gonads did not show clear cut maturity stages. Bennet (1967) claims that the sexual cycle is adjusted in both sexes so that the spawning acts coincide. A knowledge of the seasonal gonadal cycle of one sex should therefore, be quite sufficient to indicate the general pattern. Further, although *M. cupanus* fits into category C of the classification of fish on the basis of spawning activity by Prabhu (1956) and Karekar and Bal (1960), and into category II of the classification of Qasim and Qayyum (1961), Qasim's (1973) opinion that there is no definite pattern in time and duration of spawning according to which fish can be grouped seems applicable here, since neither categorization covers all contingencies in breeding.

In so far as the onset of maturation and the synchronization of spawning are concerned, although considerable literature is available to show that endogenous factors are most important (Liley 1969), in tropical waters, as stated by Antony Raja (1972), Qasim (1973) and Peter and Hontela (1978), climatic changes associated with the monsoon rains may also stimulate spawning as shown by the relation between gonado-somatic index and rainfall (figure 3). The inundation of low lying areas consequent on the monsoon floods which interconnect adjoining stretches of water and bring together large numbers of breeding pairs may also be responsible. Whatever the reason, the monsoon spawning peak leads to an increase in fish population during this season, coinciding with the peak incidence of mosquito larvae (National Filariasis Control Prevention Unit—personal communication). This indicates the larvivorous potential of the fish.

Considering fecundity, although work on Indian marine fish is voluminous, those on freshwater fish are comparatively few. Absolute fecundity has been usually related to fish or gonad length and weight (Bagenal 1966). Length has an advantage over other factors in that the fish does not shrink significantly though it can lose weight during the spawning season (Bagenal 1967). The close relationship between absolute fecundity and length demonstrated here is supported by Jhingran (1961), Mathur (1964) and Bhatt *et al* (1977), among others. Nevertheless, a variability in fecundity even in fish of the same length is noted, as stated by Le Cren (1951), Rao (1967) and Thomas (1969). This has been attributed to atresia by Henderson (1963). Winters (1971) suggests that fish, previously spawned, have a greater fecundity than fish of the same age and size spawning for the first time.

Analysis of the fecundity-weight relationships reveals that both gonad weight and body weight are exponentially related to fecundity. This could be explained by Bagenal's (1967) contention that either larger ovaries produce larger and fewer eggs or the connective tissue increases disproportionately in the ovaries of the larger fish. However, these relationships must be qualified by the fact that in many fish, somatic weight changes significantly towards the spawning period (Wootton 1973) resulting in an alteration of the relation between fecundity and weight as the season progresses. Further, a spurious correlation may be obtained when total weight is utilised since the greater number of eggs in the more fecund fish will weigh more than those in less fecund fish (Bagenal 1978).

For a better comparison of fecundities and to eliminate the alteration in (absolute) fecundity with fish age and size, the relative fecundity was calculated. The negatively linear relationships worked out in *M. cupanus* conform in some degree, to the findings of Bhatt *et al* (1977).

Connecting egg size and fecundity with the conditions for incubation, *M. cupanus* with its relatively high fecundity and rapid development exhibits parental care until the young attain a certain size (Jacob 1981). Although inclining towards 'r-selection' (Mac Arthur and Wilson 1967), the fish could be considered as a compromise on the continuum between the two extremes of 'r' and 't-selection'.

Thus the reproductive behaviour is geared to take advantage of environmental and other factors which offer the greatest opportunities for survival and development of the new generation.

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*Not referred to in the original

The germ cell types and spermiogenesis in rose-ringed parakeet *Psittacula krameri* (Scopoli)

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Abstract. By employing haematoxylin-eosin, iron haematoxylin, PAS and Feulgen reaction techniques, a morphological study has been made of spermatogenic cells in sexually matured parakeet *Psittacula krameri*. Type-A, intermediate and B spermatogonia occur in addition to type Ao (stem spermatogonia). The Ao spermatogonia is the major constituent of the testis during the regression period. Types A and B are further subdivided on the basis of nuclear size and appearance. The leptotene, zygotene, pachytene and diplotene are also differentiated into early and late stages. The spermatocytes at metaphase have also been observed but their number is very few indicating their very short life period. The round spermatids have also been subdivided into two types (i) spermatids with distinct chromatin (ii) spermatids with homogenously stained nuclei. Spermiogenesis has been subdivided into seventeen stages. The study will serve as a fundamental tool in establishing the correlation between gonadotrophins and seasonal reproductive cyclicity of the parakeets.

Keywords. Parakeet; spermatogenic cells; spermiogenesis; *Psittacula krameri*.

1. Introduction

A thorough knowledge of germ cell types and spermiogenesis is necessary for better understanding the cycle of seminiferous epithelium. In this regard, most of the mammalian species including man have been repeatedly investigated by many workers (Roosen-Runge and Giesel 1950; Leblond and Clermont 1952; Clermont and Leblond 1953; Clermont 1954, 1958, 1960, 1969, 1972; Ortavant 1954, 1956; Guraya and Bilaspuri 1976; Osman *et al* 1979; Schulze 1979). Some scanty information is available on spermatogenic cells of avian species (Schöneberg 1913; Riley 1937; Miller 1938; Mehrotra 1951; Clermont 1958; Yamamoto 1964; Yamamoto *et al* 1967; Lofts and Murton 1973; Gunawardana 1977; Yasuzumi and Yamaguchi 1977) but no detailed work seems to have been carried out on the spermatogenic cells of subtropical wild bird species. The present study was, therefore, initiated on rose-ringed parakeet *Psittacula krameri*, a common harmful bird of Punjab to have a detailed account of spermatogenic cells.

2. Materials and methods

Testes from mature parakeets (with collar) which were shot from the Punjab Agricultural University Campus, Ludhiana during the breeding period (February-April) (Dang and Guraya 1978) were used for the study. The material was fixed in alcoholic

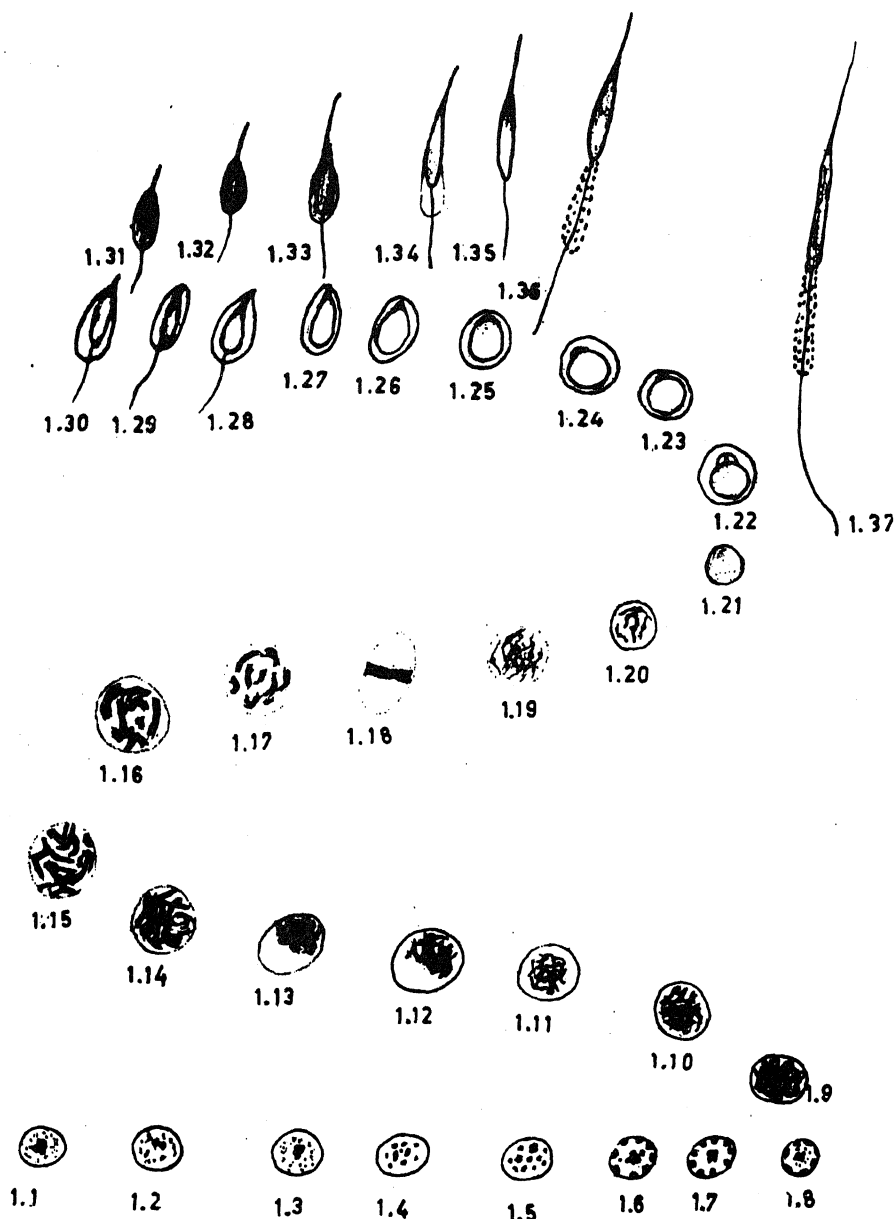


Figure 1. Different spermatogenic cells and spermiogenesis in rose-ringed parakeet, *Psittacula krameri*, 1.1, 1.2, 1.3, 1.4 = Ao, A₁, A₂, A₃ spermatogonia; 1.5 = Intermediate (In) spermatogonium; 1.6, 1.7, 1.8 = B₁, B₂, B₃ spermatogonia; 1.9, 1.10 = spermatocytes in preleptotene (PL) and leptotene (L) stage; 1.11, 1.12, 1.13 = spermatocytes in early zygotene (EZ), zygotene (Z) and late zygotene (LZ) stage; 1.14, 1.15 = spermatocytes in early pachytene (EP) and pachytene proper (P) stage; 1.16, 1.17 = spermatocytes in early diplotene (ED) and late diplotene (LD) stage; 1.18 = spermatocyte during metaphase (M); 1.19 = secondary spermatocyte (SS); 1.20, 1.21 = early spermatid (ES) and late spermatid (LS); 1.22-1.24 = spermatids during Golgi phase (GP); 1.25-1.27 = spermatids during head capping phase (HCP); 1.28-1.30 = spermatids during acrosomic phase (AP); 1.31-1.37 = spermatids during maturation phase (MP).

Bouin's fluid and Zenker-formol, dehydrated in ethanol, cleared in benzene/xylene, embedded in paraffin wax and sectioned at 7 μ . The sections were stained with (i) 0.5% Heidenhan's iron haematoxylin (mordanting in 4% iron alum and differentiation in 2% iron alum), (ii) Delafied's haematoxylin/eosin (iii) Feulgen reaction (iv) PAS-haematoxylin. Sections stained with Feulgen and haematoxylin/eosin were used for identifying different spermatogenic cells on the basis of their nuclear morphology and diameter. The spermiogenesis was studied from PAS-haematoxylin stained sections and smears. These were observed under light microscope and the diameter of the nuclei up to spermatids was measured with an ordinary micrometer. The nuclear volume of the different cells was also calculated.

3. Observations

Stained sections and smears of testes collected during February-April (breeding period) and July-September (regression period) were used. Various types of spermatogenic cells in *Psittacula krameri* have been distinguished on the basis of their nuclear morphology and diameter (table 1, figure 1).

3.1 Morphology of spermatogenic cell types

3.1a Spermatogonia: Mainly three types of spermatogonia having relatively little cytoplasm are observed during the breeding season. These are designated as types A, B and intermediate. Ao spermatogonia (stem spermatogonia or reserve stem cells) have also been identified. They generally lie in close association with basement membrane of seminiferous tubules during the breeding season. They may also lie away from basement membrane and form one or two layers in regressed testis (figure 5). They have round nuclei with less diameter as compared to other A-type spermatogonia (table 1, figures 1-5 and 7).

3.1a(i) A-type spermatogonia: A-type spermatogonia have a clear nucleoplasm with fine chromatin in the form of granules and filaments. They are of two types, either round or oblong. Depending upon their nuclear diameter and chromatin appearance, they have been classified as A₁, A₂ and A₃ (table 1, figures 1.1-1.4 and 2). The round A-type spermatogonia may be distributed either near or away from the basement membrane whereas oblong type are closely associated with basement membrane (figure 6). The long axis of oblong type spermatogonia may or may not be parallel to the basement membrane. The total volume decreases from A₁ to A₃ (table 1).

3.1a(ii) Intermediate type (In) spermatogonia: The nuclear volume and circumference of In-spermatogonium is less as compared to the A-type spermatogonia (table 1). Its chromatin is more coarser and nuclear envelope stains relatively more intensely as compared to that of A-type spermatogonia (figures 2 and 4).

3.1a(iii) B-type or Crust like spermatogonia: B-type spermatogonia are distinguished from other types by their thick nuclear envelope resulting from the deposition of chromatin crusts. They have been subdivided into B₁, B₂ and B₃ depending upon their nuclear diameter (table 1, figure 3). The nuclear diameter decreases from B₁ to B₃.

The nuclear size decreases from A-type to B-type spermatogonia (table 1) but the

Table 1. Nuclear diameter, total area and circumference (in μ) of various spermatogenic cells in rose-ringed parakeet.

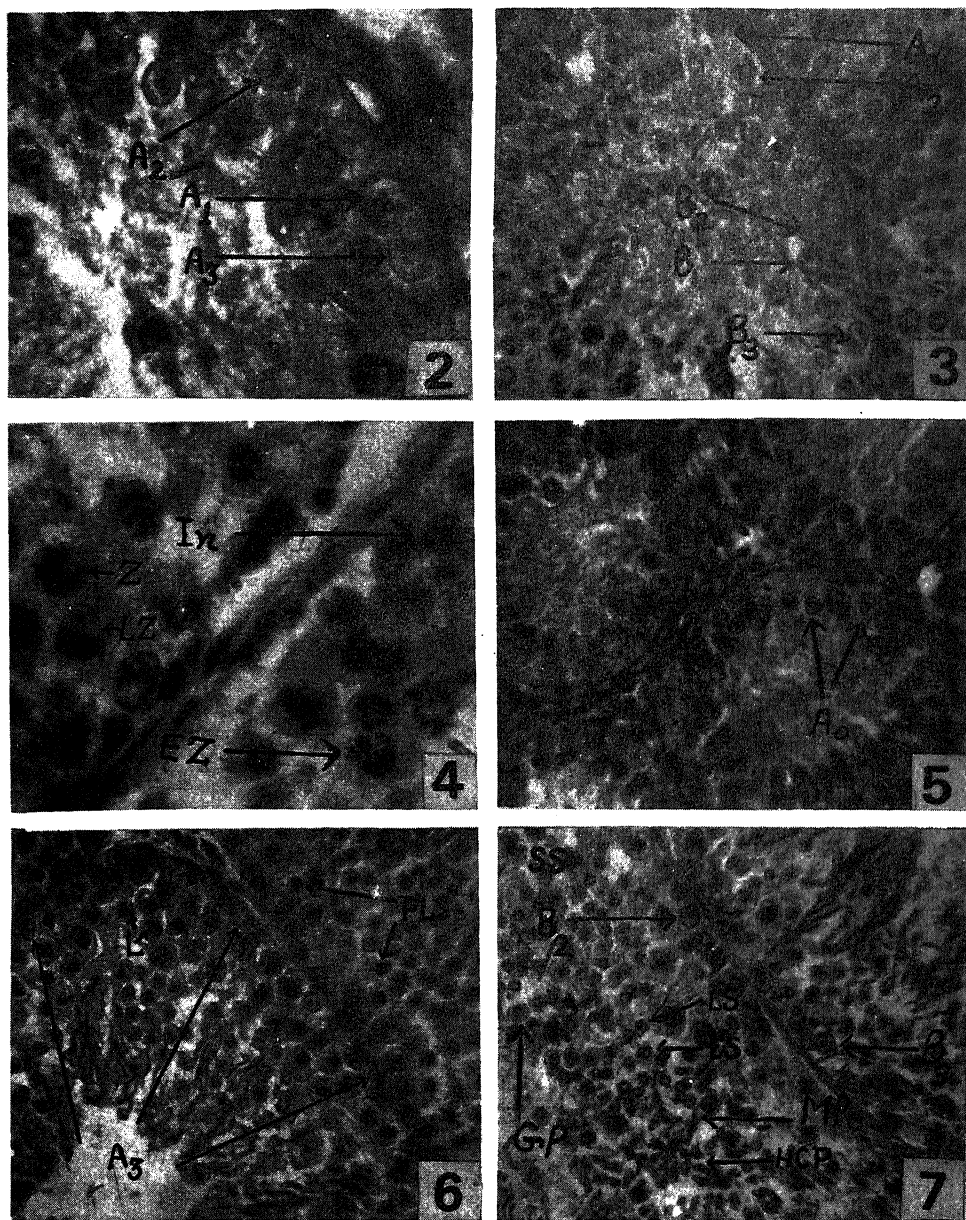
Spermatogenic cells	Diameter	Total Nuclear area (πr^2)	Total Nuclear circumference ($2 \pi r$)
<i>Spermatogonia</i>			
Ao	4.308	14.581	13.540
A ₁	5.310	22.070	16.657
A ₂	5.126	20.597	16.092
A ₃	4.909	18.860	15.401
In	4.759	17.788	14.954
B ₁	4.826	18.330	15.181
B ₂	4.250	14.125	13.326
B ₃	3.757	11.084	11.805
<i>Spermatocytes</i>			
Early preleptotene	6.128	29.507	19.261
Late preleptotene	6.613	34.352	20.781
Leptotene	7.047	39.009	22.146
Early zygotene	7.247	41.255	22.774
Late zygotene	7.297	41.826	22.931
Early pachytene	7.749	47.169	24.351
Pachytene	7.849	48.395	24.666
Early diplotene	8.049	50.893	25.295
Diplotene	8.099	51.528	25.452
Metaphase	8.684	59.255	27.294
<i>Secondary spermatocyte</i>			
Spermatids	5.177	21.050	16.267
Early round spermatid	3.423	9.201	10.755
Late spermatid before start of spermiogenesis	2.789	6.107	8.763

chromatin concentration correspondingly increases from reserve stem cell (Ao) to B₃ spermatogonia (figures 1.1-1.8, 2, 3 and 4).

3.1b *Spermatocytes*

3.1b(i) *Primary spermatocytes*: The B-type spermatogonia enter preleptotene and then meiotic prophase. On the basis of nuclear morphology and diameter, the preleptotene is distinguished into early and late preleptotene. The early preleptotene spermatocytes with jumbled chromatin are darkly stained as compared to late preleptotene spermatocytes with distinct chromatin (figures 1.9, 1.10 and 6). The nuclear size increases from preleptotene to late preleptotene (table 1). The meiotic prophase in rose-ringed parakeet consists of usual stages of leptotene, zygotene, pachytene, diplotene and diakinesis. The nuclear chromatin and volume changes are shown in figure 1.10-1.18 and table 1. The different stages have been differentiated into early and late stages.

3.1b(ii) *Secondary spermatocytes*: The secondary spermatocytes nuclei are small as compared to the preceding spermatocytes (table 1, figures 1.16, 1.19, 4 and 7). The nuclei have distinct chromatin with heavily stained nuclear membrane. They are occasionally observed, indicating that they immediately divide to form spermatids.



Figures 2-7 Testis sections during breeding, regression and regeneration period showing different spermatogenic cells. For abbreviations see text of figure 1.

3.1b(iii) *Spermatids*: The secondary spermatocytes by second meiotic division form the spermatids. The immediately-formed spermatids have round nuclei with a diameter of 3.723μ with distinct chromatin (table 1, figures 1.20 and 7). Later on these spermatids become smaller and with less basophilic nuclei (figures 1.21 and 7). The different stages of this division could not be identified probably due to their short duration.

3.2 Spermiogenesis

Spermiogenesis in the parakeet has been studied from sections and smears stained with PAS-haematoxylin and haematoxylin/eosin. The detailed transformation taking place during spermiogenesis is shown in figures 1.21-1.37. It is divided into four main phases, Golgi phase, head cap phase, acrosome phase and maturation phase. The four phases are classified as follows: In the Golgi phase, the Golgi zone of the newly formed spermatids forms the proacrosomic granules which fuse to form acrosomic granule. In the head-cap phase, the acrosomic granule becomes elongated to form acrosomic system with the head cap and attaches to nucleus. In the acrosome phase, the acrosome becomes conical and nucleus is also elongated. In maturation phase, the spermatids are transformed into spermatozoa after a number of changes in the shape of the nucleus and acrosome.

The aforementioned phases of the spermiogenesis are further divided into seventeen subdivisions, mainly based on the development of acrosomic system and the change of spermatid nucleus. The Golgi phase includes steps 21-24, the head cap phase steps 25-27, the acrosome phase steps 28-30 and the maturation phase steps 31-37.

4. Discussion

Two types of spermatogonia have been described previously in birds (Schöneberg 1913; Riley 1937; Miller 1938; Lake 1956; Clermont 1958; Lofts and Murton 1973). But this study has revealed three types of spermatogonia besides stem spermatogonia in parakeets. These spermatogonia are classified as A, B and intermediate. The intermediate spermatogonia have also been identified in quail (Yamamoto *et al* 1967) and in a number of mammalian species (Clermont 1954, 1960; Oakberg 1956; Monesi 1962; De-Reviere 1971; Guraya and Bilaspuri 1976). The A-type spermatogonia are larger than intermediate and B-type spermatogonia. The early and late preleptotene stages distinguished in the parakeet have not been previously reported in birds and most of the mammalian species (Yamamoto *et al* 1967; Leblond and Clermont 1952; Clermont 1960; Ortavant 1956; Oakberg 1956; Hochereau-DeReviere 1970). However, such a distinction is made in goat (Guraya and Bilaspuri 1976). In quail, Yamamoto *et al* (1967) distinguished only leptotene, zygotene and pachytene stages of spermatocytes. Depending upon the chromatin appearance and nuclear diameter, early and late stages of preleptotene, leptotene, zygotene, pachytene, diplotene and metaphase have also been distinguished. These results cannot be compared with other bird species as no such detailed studies are made previously.

The secondary spermatocytes reported in rose-ringed parakeet have smaller nuclei than the preceding spermatocytes and are very few in number. Similar results have been reported in quail (Yamamoto *et al* 1967).

Spermiogenesis has been grouped into four phases, Golgi phase, head cap phase, acrosomic phase and maturation phase as reported previously in birds and mammals (Leblond and Clermont 1952; Clermont 1954; Ortavant 1954; Clermont 1960; Kramer 1960; Hochereau 1963; Swierstra and Foote 1963). Spermiogenesis has been divided into different subdivisions in different species. Twelve subdivisions have been reported in quail (Yamamoto *et al* 1967), ten in domestic fowl (Gunawardana 1977), fifteen in rooster (Cavazos and Melampy 1954). However, De-Reviere (1971) opined that PAS staining is not a satisfactory technique in the fowl for the recognition of spermatid stages. Clermont (1958) also observed that PAS staining becomes

weaker at stage seven in duck spermatids. The present authors also believe that only PAS technique is not sufficient to trace all the subdivisions of spermiogenesis. The seventeen subdivisions of spermiogenesis in rose-ringed parakeet have been observed from the sections and smears stained with PAS-haematoxylin, iron haematoxylin and haematoxylin/eosin.

5. Conclusions

In most mammalian species, definite cellular associations can be recognised at any given point in the seminiferous epithelium and these clearly indicate a seminiferous epithelial cycle. Such definite associations have not been reported in birds. Perhaps this is due to lack of efforts for a detailed study of kinetics of spermatogenesis. The present investigation will form the basis for future quantitative analysis of the effects of climatic factors and antifertility compounds on spermatogenesis in birds. It will also help in the study of seminiferous epithelium cycle and of the relationship between gonadotropins and spermatogenic activity.

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Synchronous development of the ovary and the female accessory sex glands of a crustacean, *Squilla holoschista*

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Abstract. Based on the histology of the female accessory sex glands (cement glands) and the sequential appearance of their secretory products, four stages have been distinguished in the development of the cement glands in *S. holoschista*. This paper describes the various stages in ovarian development and correlates them to the cement glands activity. A further correlation between the ovarian and cement glands activity has been shown by statistical analyses. A possible endocrine mechanism regulating the synchronous activity of the ovary and cement glands is proposed.

Keywords. Female accessory sex glands (cement glands); Ovary; synchronous activity; *Squilla*.

1. Introduction

In recent years, much attention has been paid to the study of accessory sex glands of arthropods (Adiyodi and Adiyodi 1974). Many male insects utilise their secretion for spermatophore formation, sperm maturation, activation and transportation, formation of mating plug, egg maturation, oviposition, alteration of the mating behaviour, and also for production of fecundity-enhancing and receptivity-inhibiting substances (Leopold 1976; Gillot and Friedel 1977). Among arachnids, the Scorpionidea and the Pseudoscorpinoidea possess complex accessory reproductive glands, whose main function in the male is to form the chitinous spermatophore. Accessory sex glands of the crustacea have not been studied in any great detail. Specialized male accessory sex glands have been described from three crustacean groups (Fryer 1960; Wolfe 1971; Deecaraman and Subramoniam 1980a). Some female crustaceans possess cement glands which secrete a dense mucoid substance necessary for agglutination and also for holding the eggs together after laying (Yonge 1937; Lloyd and Yonge 1940; Mason 1970).

In some decapods, Lloyd and Yonge (1940) reported synchronous development of the ovigerous setae and ovary. A relationship between cement glands development and ovarian maturation is known to exist in crayfishes (Stephens 1952). In *S. mantis*, for instance, Do-chi (1975) recorded a cyclic activity of the cement glands correlated to vitellogenesis. Recently, Deecaraman and Subramoniam (1980b) reported four stages in the activity of the cement glands of *S. holoschista*. This paper deals with the developmental stages of the ovary in relation to the developmental stages of female accessory sex glands (cement glands) in *S. holoschista*.

2. Materials and methods

S. holoschista, used in the present study, was collected from the Madras coast. For routine histological studies, the ovaries and cement glands were fixed in Bouin's fluid and 10% neutral buffered formaldehyde. Sections (6 μm thick) were stained in haemotoxylin of Delafield, Ehrlich, Mallory's and Mason's.

From histological and squash preparations, the cement glands and ovaries were classified into four stages depending upon their size, and the presence or absence of cytoplasmic inclusions. The oocytes and cement glands were measured taking the average of their longest and shortest axes. For each stage of the ovarian and cement glands development, 50 measurements were made and the average calculated and plotted in the histogram. The data obtained were subjected to statistical analyses.

3. Results

The cement glands are found as dense white patches in the mature female on the ventral side of the sixth, seventh and eighth thoracic segments. In the spent female, the cement glands are shrunken and not visible externally. To follow the sequential changes in the structure and chemical composition of the cement gland, its growth was divided into four stages (Deecaraman and Subramoniam 1980b), a categorization also used in the present study.

3.1 Ovarian development stages

The ovarian development of *S. holoschista* has been arbitrarily divided into four stages based on oocyte diameter and histological characteristics, which compared well with developmental stages of the cement glands.

3.1a Stage I: In this stage, the ovary is thin, fragile and white-to-cream coloured, with black pigments over its surface. Each oocyte measures 8.58 μm in diameter and is elliptical in shape. The nucleus (4 μm across) occupies a major portion of the oocyte. The ooplasm which is scarce forms a thin rim over the nucleus. The chromatin granules are found towards the inner surface of the nucleus (figure 1). This stage corresponds to the first developmental stage of cement glands.

3.1b Stage II: The ovary is faint yellow in colour. The black pigmentation which was conspicuous over the surface of the ovary in stage I is now much reduced. The oocytes are larger (27.12 μm) in diameter but the nucleus is smaller (3 μm). The ooplasm now occupies more space than the nucleus and is provided with yolk granules. The chromatin granules are still visible. This stage corresponds to the second stage of development of the cement glands.

3.1c Stage III: The ovary which is yellow and large extends profusely on either side of the thoracic and abdominal segments and forms lateral diverticulae. The oocyte measures 45.62 μm in diameter; the nucleus is very much reduced and measures only 2 μm . At this stage, the oocytes lose their spherical shape; the ooplasm occupies more space and is filled with yolk granules and globules. This stage is comparable to the third stage of development of the cement glands.

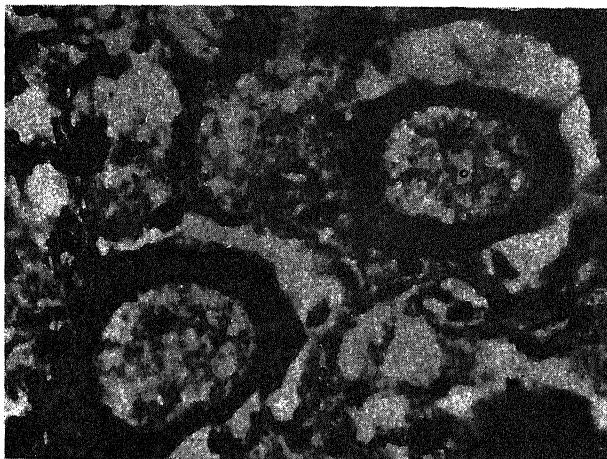


Figure 1. Transverse section of the ovary to show the oocytes at stage I. Stained in Ehrlich's haematoxylin ($\times 1125$).

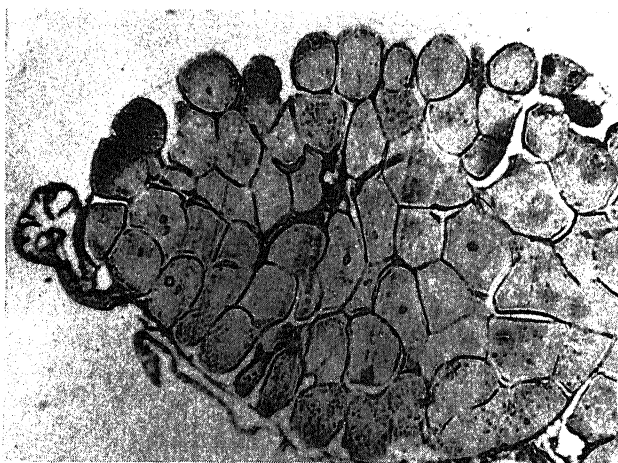


Figure 2. Transverse section of the ovary to show the oocytes at stage IV. Stained in Ehrlich's haematoxylin ($\times 250$).

3.1d Stage-IV: The ovary is bright golden yellow; the oocytes measure $53.42 \mu\text{m}$ and the nucleus only $2 \mu\text{m}$ in diameter (figure 2). The cytoplasm is filled with yolk granules and globules. This stage corresponds to the fourth stage of development of the cement glands.

The data obtained from the developmental stages of ovaries and cement glands were subjected to statistical analyses. The analysis of variance (*f*-tests) reveals significant growth between all the stages of oocytes as well as cement glands (table 1). Table 2 shows that growth in the means of the oocyte as well as cement glands for any pair of stages is significant, suggesting synchronous development of both the organs (figure 3).

Table 1. Growth in the stages of oocytes and cement glands (50 samples of each stage).

Material	Stages				
	I	II	III	IV	
Mean and standard deviation.					
Oocytes		8.58±1.787	27.12±2.312	45.62±6.786	53.92±5.791
Cement glands	A	14.38±1.925	20.92±1.432	24.24±3.240	35.05±4.668
	B	13.64±4.500	16.72±2.940	32.16±2.962	29.52±2.844

Value of f observed: oocytes, 6.975; cement glands A, 233.24; cement glands B, 290.55;

Value of f calculated in all cases was 2.67.

Table 2. Showing further significant growth of oocytes and cement glands.

Oocytes stages		t (observed)
A	Stage first oocytes	20.4674
B	Stage second oocytes on third	15.0231
C	Stage third oocytes on fourth	5.5063
Cement glands stages		
A	Stage first A type gland on second	11.731
B	Stage second A type gland on third	3.7935
C	Stage third A type gland on fourth	11.4905
D	Stage first B type of gland on second	3.9039
E	Stage second B type of gland on third	9.9203
F	Stage third B type of gland on fourth	13.050

Value of t (expected) in all cases was 1.96.

4. Discussion

The present study has shown a relationship between stages in development of the ovary and stages in the activity of cement glands, suggesting a close synchrony in the function of these two organs in *S. holoschista*. Similar synchrony in development of the cement glands and ovary has been reported in crayfish and *S. mantis* by Lloyd and Yonge (1940), Stephens (1952) and Do-chi (1975). Experiments involving eyestalk ablation and thoracic ganglia extract injection have indicated hormonal influences in development of the ovary and cement glands in *S. holoschista* (Deecaraman and Subramoniam 1979). Identical results were also obtained by Stephens (1952) in different species of *Procambarus*. In the Crustacea, the ovarian hormone influences the appearance of permanent as well as temporary (blood-related) secondary sexual characters (Lloyd and Yonge 1940; Adiyodi and Adiyodi 1970; Mason 1970). Further,

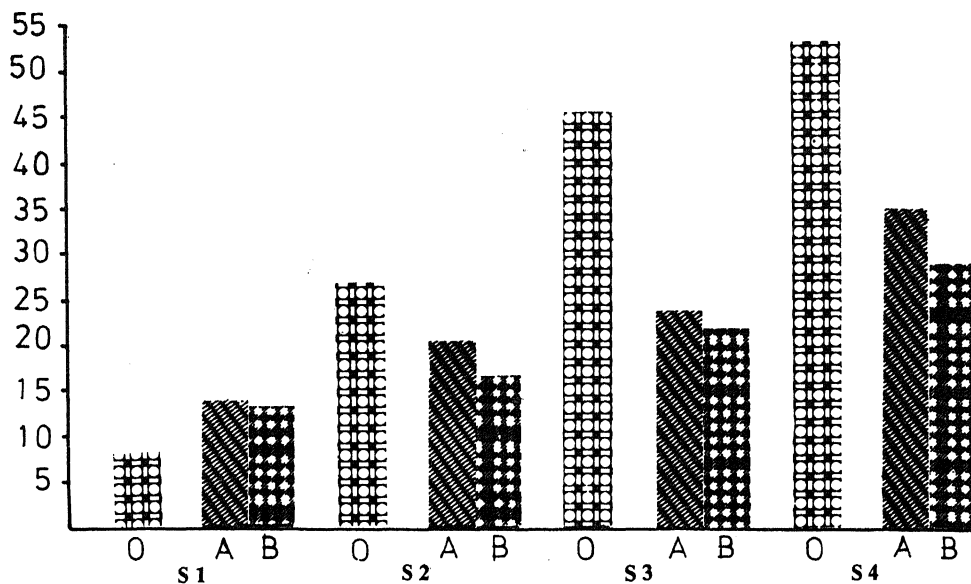


Figure 3. Histograms representing comparative developmental stages of oocytes and A and B type of cement glands. Stages—1, 2, 3 and 4.

these temporary secondary sexual characters appear only during the breeding season as they are apparently under the control of a hormone produced by the maturing ovary (Adiyodi 1978). Activity of cement glands in stomatopod crustaceans, correlated to ovarian maturation may be considered as a temporary secondary sexual character (Deecaraman 1980c). But in view of the parallel development of the ovaries and cement glands it is unlikely that an ovarian hormone may be involved in the initiation of cement gland development. The synchronous development of the ovaries and cement glands in *S. holoschista* suggests that endocrine regulatory mechanisms exist that serve to correlate cyclic development of the ovaries and cement glands.

Acknowledgements

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Aggressive behaviour of captive *Bandicota bengalensis*

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Abstract. The aggressive behaviour rhythm of *Bandicota bengalensis* under different social conditions were studied in the laboratory. The number, intensity and duration of aggressive behaviour peaks varied depending on the number, sex and social status of the interacting conspecific/s. Significantly the peaks of aggression were recorded during the dark period. In heterosexual conflicts both sexes were equally belligerent. However in male interactions with more females, the former emerged dominant always. Similarly one male dominated another in male-male encounters. Confrontation amongst several males in the presence of female indicated the existence of only *alpha* and *omega* males.

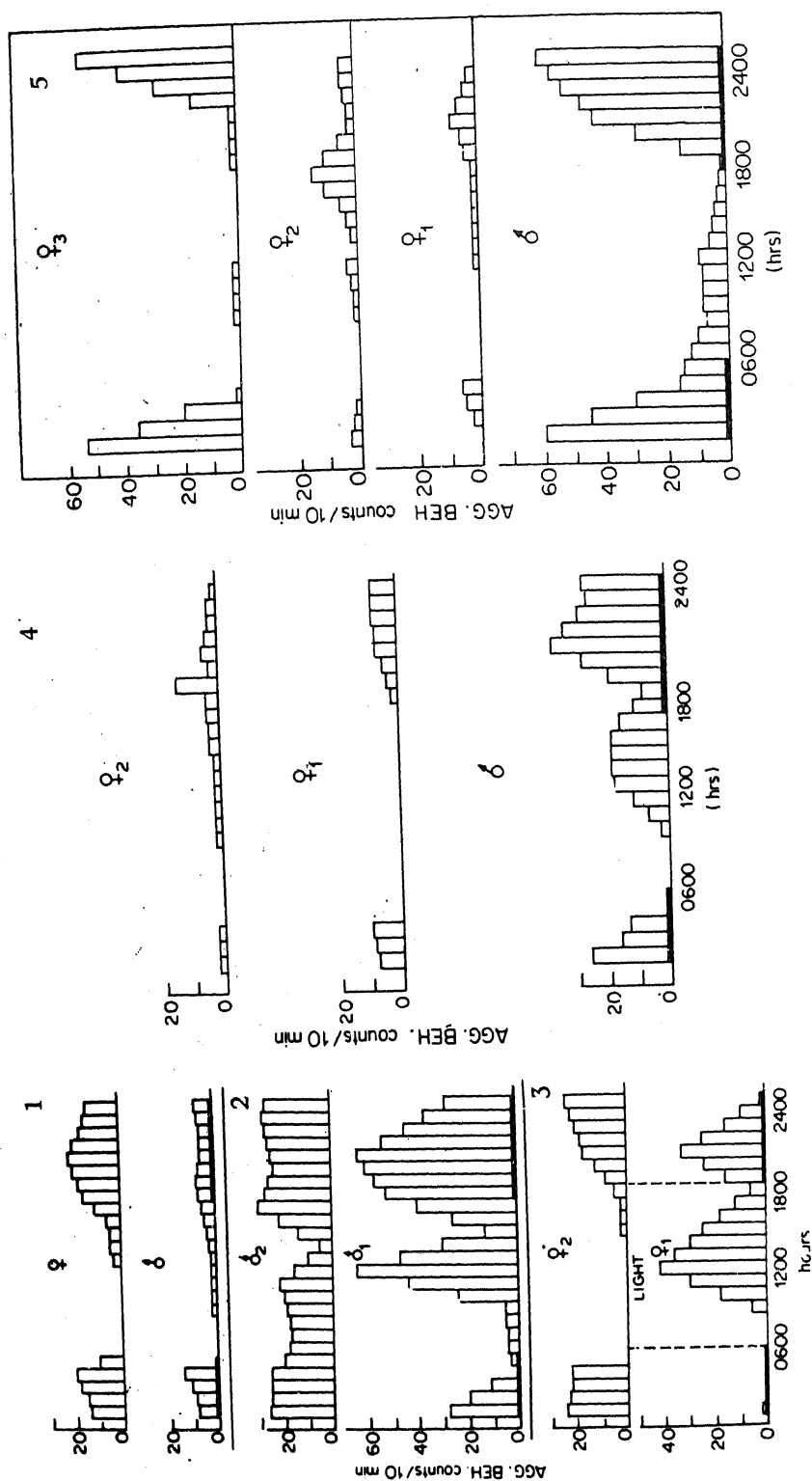
Keywords. *Bandicota bengalensis*; aggressive behaviour; male dominance; *alpha* males; *omega* males.

1. Introduction

Circadian rhythms of rodents have been well documented for behaviour under natural conditions (Marten 1973), for activity movement in cages and mazes (Barnett *et al* 1975), for feeding and drinking (Possidente and Birnbaum 1979) and for aggressive behaviour (Landau 1975; Lerwill 1977). Desynchronization of both metabolic and behavioural rhythms of *Peromyscus maniculatus* occurred due to social stress (Farr and Andrews 1978). Similar dissociation of activity rhythms due to crowding has been observed by several others (Calhoun 1975; Nygren 1978). In this article we report alterations in the rhythms of aggressive behaviour of lesser bandicoot rat, *Bandicota bengalensis* under different social situations.

2. Material and methods

Bandicota bengalensis, weighing 200-500 g were housed singly in metal cages (35 × 35 × 50 cm) and were fed on rat and mouse feed (Hindustan Lever, India) supplemented with vitamins and vegetables once a week with *ad lib* supply of water. Photoperiod was regulated at 12 hr light and 12 hr darkness with the light period beginning at 0600 hours. For recording behaviour the animals were transferred to an observation chamber, details of which have been described earlier (Sridhara and Krishnamoorthy 1982). Aggressive behaviour of individual members during encounters between male-female, male-male, female-female, one male-two females and one male-three females were studied. Attempts to study agonistic behaviour in groups involving more than one male were unsuccessful as submissive males died within 24 hr after being grouped with dominant males. One member of the pair/male in pair/group combination was restricted to one portion of the chamber with the other portion housing the



Figures 1-5. Each histogram represents the oscillation of an interacting individual in the cage over a 24 hr period.

second member / females. By removing the partition the animals were allowed to interact for 10 min during each hour of the LD cycle. The aggressive behavioural acts and postures namely threat, crawling under / over lunge, chase, attack, fight, boxing and bite (Grant and Mackintosh 1963) were scored for each member of the pair / group during these observations. The data were collected under natural daylight during day time and under dim red light during nights. Four replications were carried out for each type of encounter over a seven day schedule and histograms were computed from the combined means of different individuals. The aggressiveness of either sex was established by student *t* tests. To recognise ranks of aggressive behaviour, if any, during group encounters, Kruskal-Wallis one-way analysis was carried out (Siegel 1956).

3. Results

The results presented in figures 1-5 illustrate that aggressiveness of bandicoots, motivated through the presence of other conspecifics, is rhythmic. The rhythmicity is either bimodal or unimodal depending on the sex, status and number of interacting conspecifics. However, a 7-day long study indicated that the animals have shown a fairly consistent rhythmicity (95% rhythms) in their behaviour. Males exhibited a single peak at 0400 hr in male-female encounters (figure 1), at 0100 hr in one male-three females interaction (figure 5), but two peaks of equal intensity at 1200 and 2100 hr in male-male confrontation (figure 2) and three peaks at 0100, 1300 and 2100 hr in one male-three females group encounter, the last mentioned peak being the most intensive (figure 4). The second male in male-male pair exhibited a single peak of aggressive behaviour at 1700 hr (figure 2). The female in male-female pair and one of the females in the female-female encounter displayed two peaks of aggression, at 0400 and 2100 hr in the former case (figure 1) and at 1300 and 2200 hr in the latter (figure 2). Maximum aggressive behaviour was scored for the second female of the female-female (figure 2) and third female of one male-three females encounter at 0100 hr (figure 5). One female of one male-two females conflict exhibited peak aggressive behaviour at 0400 hr while the second female shifted her intensity to 1900 hr (figure 4). Similarly the first and second females of one male-three females interaction spaced out their peak aggressive behaviour rhythm at 2200 and 1800 hr (figure 5).

Majority of peaks for aggressive behaviour occurred during the dark phase. Only one of the males of the male-male pair and one of the females of the female-female pair displayed afternoon peaks while the other male of male-male pair and second female of one male-three females interaction displayed intensive aggressive behaviour at twilight hours.

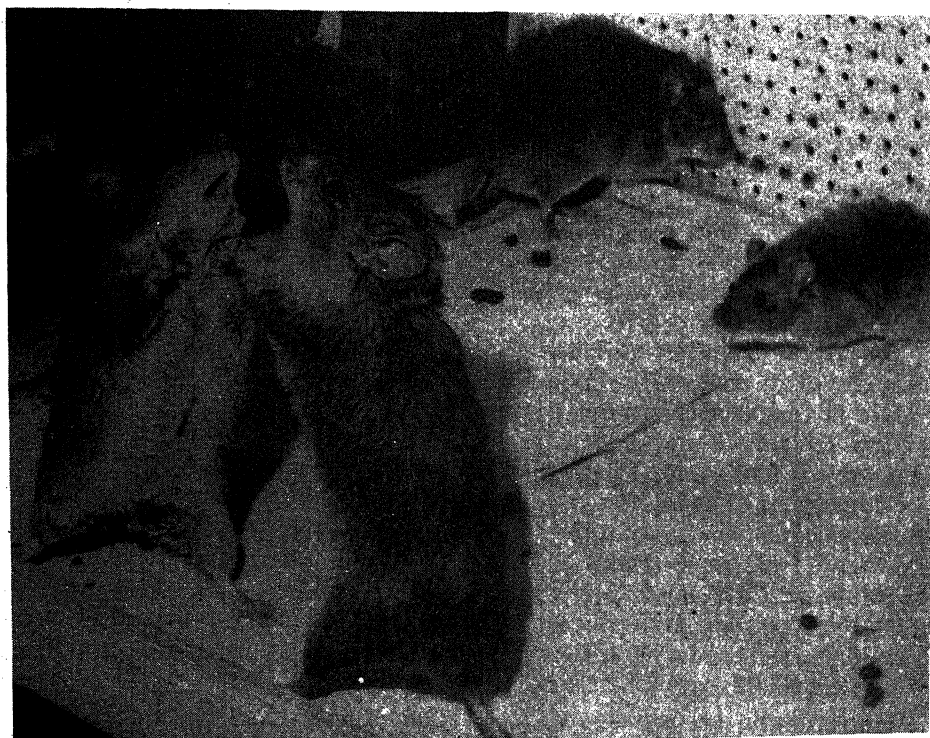
Comparison of aggressive behaviour between the sexes indicated male and female as equally belligerent ($P < 0.05$, table 1) in male-female clashes while in male-male and female-female clashes, one of them established dominance over the other ($P < 0.01$ and < 0.02 , table 1). The male was significantly more aggressive than females in encounters involving more than one female ($P < 0.01$, table 1). No rank order of aggressiveness could be established in the two group clashes ($H = 3.021$ and 4.32 , $P < 0.104$).

Confrontation ensued between two equally matched males immediately after their placement in the observation chamber (figure 6), continued violently (figure 7) till one of them was defeated and assumed submissive posture (figure 8). The latter, though not wounded fatally, succumbed to death within 24 hr. Thus we

Table 1. Comparison of aggressive behaviour counts scored by two sexes during different social interactions.

Confrontation between	Scored by	Mean score $\pm$ S.E.	Cumulative score for the combination
M and F	M	120 $\pm$ 28	353
	F	233 $\pm$ 37	
M and M	M 1	741 $\pm$ 51*	1255
	M 2	514 $\pm$ 35	
F and F	F 1	446 $\pm$ 48*	619
	F 2	173 $\pm$ 26	
1 M and 2 F	M	358 $\pm$ 29*	482
	F 1	66 $\pm$ 8	
	F 2	58 $\pm$ 6	
1 M and 3 F	M	487 $\pm$ 37*	639
	F 1	58 $\pm$ 16	
	F 2	73 $\pm$ 14	
	F 3	21 $\pm$ 23	

M = male; F = female; * Significantly higher aggressive behaviour counts.

**Figure 6.** Both male rats are equally aggressive. The one crouching in the corner is *omega*.

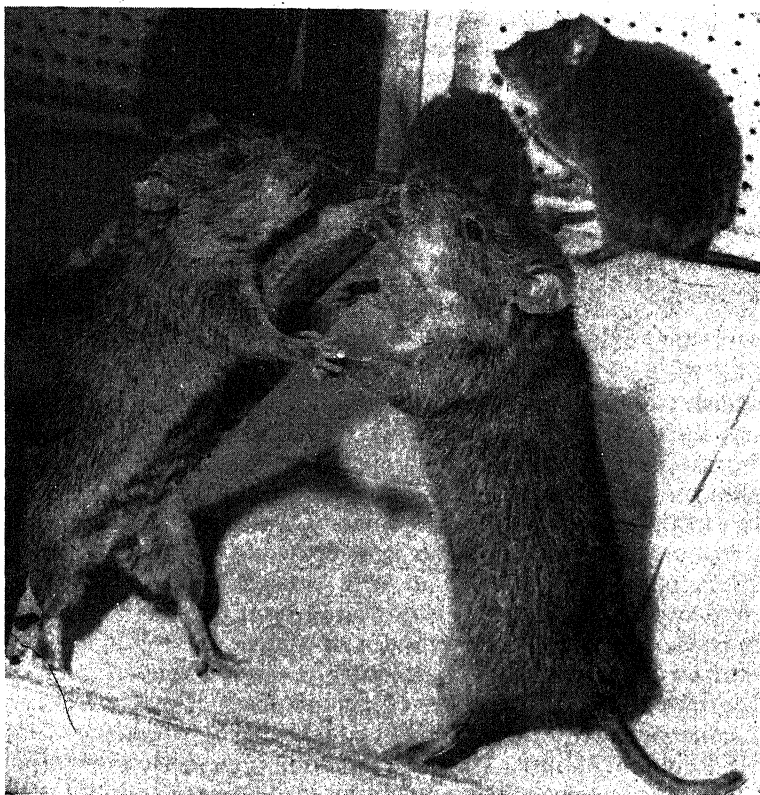


Figure 7. 'Boxing' posture of the conflicting rats. Submissive posture by an *omega* male in the background.



Figure 8. The male on the right is *alpha*; the one on the left is displaying submissive posture and is *omega*. Another *omega* is crouching in the background.

can assume the winner and loser of this encounter as equivalent to *alpha* and *omega* of *Rattus norvegicus* (Barnett 1975). The third male crouching in a corner (figures 6-8) displayed submissive posture even in the absence of threat/attack and avoided the clashing males, but still died without even being attacked, thus also qualifying for *omega* status. No *betas* were identified, which could be either due to the unnatural cage conditions of experimentation or due to the extreme violence of *alpha B. bengalensis* which does not allow other males to survive in his presence.

4. Discussion

Parrack (1966) noticed two peaks of locomotor activity in *B. bengalensis*, one soon after the dark phase and the other early in the morning. Spillet (1968) failed to establish any rhythm for the agonistic behaviour in grouped *B. bengalensis* although such activity increased between 0200 and 0530 hr. However, Parrack and Thomas (1970) noticed aggressive peaks for dominant *B. bengalensis* at 0600 and 0900 hr. The occurrence of nightly peaks of aggression may be a natural corollary of the enhanced general activity of this nocturnal species during dark phase. The present results illustrate both rhythmic and non-rhythmic circadian oscillations of aggressive behaviour of *B. bengalensis*, which are not stable but are influenced by the number, status and sex of the interacting conspecifics. Similar phase dissociation of metabolic and behavioural rhythms of *P. maniculatus* occurred when intraspecific interactions were enhanced by crowding (Farr and Andrews 1978). Such failure of maintenance of phase relationships was drastic in subordinate animals which could be due to avoidance of more aggressive and dominant cohorts. The great variation seen in the aggressive behaviour rhythms in the present study is a reflection of social interactions/adjustments in the different stress situations of the experiments.

The social organization of lesser bandicoots differs from that of *Rattus norvegicus* in that the females are also aggressive in heterosexual paired encounters. Males can be categorised into *alpha* and *omega* as in *R. norvegicus*.

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Weight changes and survival of a tropical earthworm *Pontoscolex Corethrurus*

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Abstract. The ability of healthy individuals of *Pontoscolex corethrurus* to survive and maintain its weight in tap water and different concentrations of NaCl and sucrose solution is studied. In water, earthworms survived normally but died at 48 hr exposure in 5% sugar and 0.4% NaCl. In water, an increase of 26% in total body weight was observed at 4 hr exposure. Weight also increased when exposed to 1, 2, 3 and 4% sugar solution, but it decreased in 5% sugar solution. This decrease in weight may be due to osmotic crisis. Similar weight changes have also been observed in NaCl solutions. An increase in weight was observed up to 0.3% NaCl and a decrease in weight in 0.4% NaCl and above.

Keywords. *Pontoscolex corethrurus*; earthworm; exposure; weight changes; survival in different media.

1. Introduction

Earthworms are often subjected to changes in soil water and chemicals and have to adapt themselves to the changing conditions. Earthworms have to maintain their water equilibrium for their vital activities. Hence information on osmotic balance in tropical earthworms become imperative. Osmoregulation has been studied in terms of weight regulation in aquatic oligochaetes (Ganapathy and Subba Rao 1972) and a few semi-terrestrial oligochaetes (Báhl 1945; Oglesby 1970; Dietz and Alvarado 1970) with reference to water and different salinity levels. *Pontoscolex corethrurus*, a tropical earthworm, faces the problem of water and chemical content of the soil. Some preliminary studies on its biology (Arunachalam 1978) and distribution (Kale and Krishnamoorthy 1981) have been carried out in this species, but not on its weight regulation when exposed to adverse media. An attempt has therefore been made to investigate the ability of *P. corethrurus* to maintain its weight with reference to tap water and different concentrations of NaCl and sucrose solutions.

2. Material and methods

P. corethrurus were collected from the field and reared in the laboratory in a large glass aquarium (65 × 35 × 35 × cm) containing natural medium. Dried leaves were added to the soil to supplement the natural medium and water sprinkled to make the soil sufficiently wet. The soil temperature of the culture medium was 28 ± 1°C. From this stock, earthworms were selected for various experiments.

To find out the effect of certain adverse media on body weight, *P. corethrurus* (396 ± 106.2 mg body weight) were individually immersed in tap water, in different concentrations of sugar (1, 2, 3, 4 and 5%) and NaCl (0.05, 0.1, 0.2, 0.3, 0.4

Table 1. Survival of *P. corethrurus* when exposed to different concentrations of sugar solution at different times of exposure (No. of individuals tested: 10).

Concentration of sugar (%)	% survival at different times of exposure (hr)			
	24	48	72	96
Freshwater	100	100	100	100*
1	100	100	100	100
2	100	100	100	100
3	100	100	100	0
4	100	100	0	0
5	100	0	0	0
10	0	0	0	0

* No mortality till one month.

Table 2. Survival of *P. corethrurus* when exposed to different concentrations of NaCl at different times of exposure. (No. of individuals tested: 10).

Concentration of NaCl (%)	% survival at different times of exposure (hr)				Remarks
	24	38	72	96	
Freshwater	100	100	100	100	Normal
0.1	100	100	100	100	"
0.2	100	100	100	100	"
0.3	100	100	100	100	"
0.4	100	100	100	100	Shrunken
0.5	100	100	100	100	"
0.6	0	0	0	0	

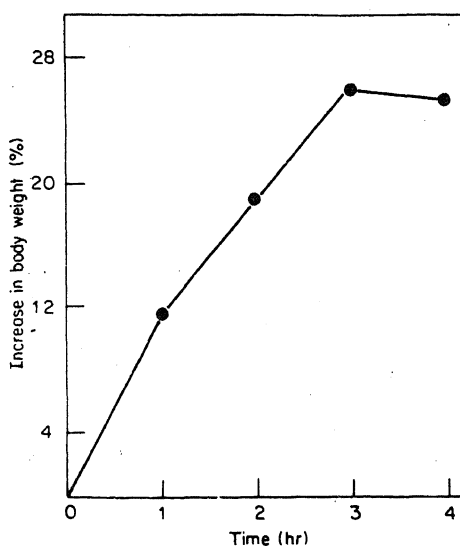
**Figure 1.** Increase in the body weight of *P. corethrurus* when immersed in water as a function of time. Each value (mean) is the average performance of five individuals.

Table 3. Effect of different concentrations of sugar on body weight in *P. corethrurus*.

Concentration of sugar (%)	Live body weight (mg)	Changes in body weight (%)			
		1 hr	2 hr	3 hr	4 hr
1	450.2± 96.78	4.6±1.23	10.4 ±2.11	19.1±2.67	21.0±4.52
2	385.7±100.21	5.0±1.18	9.97±1.97	27.8±3.88	31.3±7.48
3	416.7± 80.32	2.5±0.89	12.8±2.56	26.2±6.41	28.9±6.21
4	424.7±104.78	2.5±0.91	10.4±1.91	21.6±5.94	29.3±4.79
5	380.8± 75.32	-5.7±2.31	-10.4±2.14	-16.2±3.81	-16.9±3.41

Each value (mean±SD) is the average performance of 5 individuals (– denotes decrease in weight).

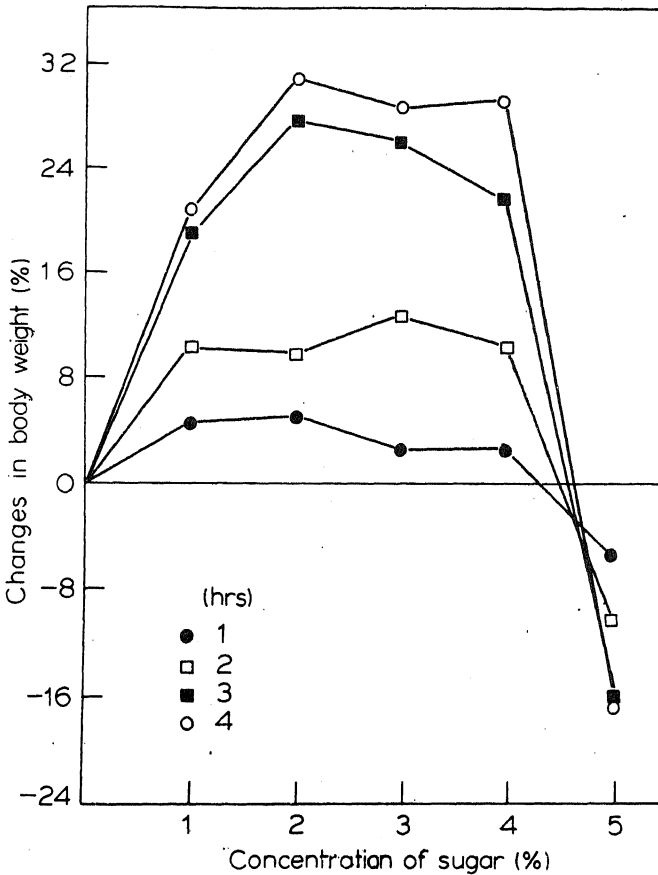


Figure 2. Changes in the body weight of *P. corethrurus* as a function of sugar concentrations. Each value (mean) is the average performance of five individuals.

and 0.5%). The individuals were exposed to different media and weighed at an interval of 1 hr for a 4 hr immersion. Suitable corrections were made in the weight of individuals for sand particles present in the gut at different times of exposure (Arunachalam *et al* 1982). The weight changes in different media at different

hourly exposure were expressed in terms of percentage of its initial live weight. Five individuals were tested in each media.

For survival studies, groups of individuals (10 in number) were exposed to tap water, different concentrations of sugar solutions (1, 2, 3, 4, 5 and 10%) and NaCl solutions (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%). The media (2L) in which the individuals were exposed was aerated continuously. The survival of *P. corethrurus* in different media was observed at an interval of 24 hr for a total period of 96 hr exposure and was expressed in terms of percentage.

3. Results and discussion

3.1 Survival in different media

When *P. corethrurus* were submerged in aerated water, no mortality was observed for one month (tables 1 and 2). Similar observations have been made in several species of earthworms (Edwards and Lofty 1977).

The effect of different concentrations of sugar solution on the survival of *P. corethrurus* in different hourly exposure are presented in table 1. For 1 and 2% sugar solutions, no mortality was observed at 96 hr exposure. In 3% and above, mortality occurred within 96 hr exposure. On increasing the concentrations, the time of exposure for 100% mortality decreased i.e., the resistance time decreased with increase in concentrations.

Earthworms exposed up to 0.3% NaCl were healthy and exhibited normal movements; but in 0.4 and 0.5% they were shrunken in their nature with feeble movements (table 2). However, they survived till 96 hr exposure. In 0.6% NaCl *P. corethrurus* did not survive even for 1 hr exposure.

These results revealed that the survival limit of sugar and NaCl concentrations were 2 and 0.3% respectively. Above these levels of sugar and NaCl, changes in the body conditions occurred causing death.

3.2 Changes in weight in different media

When *P. corethrurus* were immersed in tap water, the live weight increased as a function of time. The weight gradually increased up to 3 hr exposure and was then levelled off (figure 1). For instance, the average weight in *P. corethrurus* increased to 11.6% at 1 hr exposure, to 26% at 3 hr exposure and no further increase in weight at 4 hr exposure. This is in accordance with Wolf (1940) who reported that *L. terrestris* gained up to 15% of its total live weight in 5 hr

Table 4. Effect of NaCl concentrations on body weight of *P. corethrurus*.

Concentration of NaCl (%)	Live body weight (mg)	Changes in body weight (%)			
		1 hr	2 hr	3 hr	4 hr
0.05	380.2 ± 65.21	6.4 ± 2.10	11.5 ± 3.47	19.6 ± 4.12	22.8 ± 2.26
0.1	426.9 ± 70.18	7.9 ± 1.89	15.8 ± 3.18	22.4 ± 5.21	21.2 ± 1.12
0.2	395.2 ± 100.26	4.8 ± 1.21	9.9 ± 1.98	14.8 ± 3.17	17.2 ± 2.28
0.3	416.7 ± 98.65	4.4 ± 1.08	5.8 ± 1.04	12.0 ± 2.55	14.1 ± 2.14
0.4	383.7 ± 65.45	-0.8 ± 0.21	-1.3 ± 0.82	-4.9 ± 1.94	-7.8 ± 2.10
0.5	414.8 ± 78.65	-4.3 ± 1.24	-6.4 ± 1.28	-11.0 ± 2.78	-15.8 ± 2.52

Each value (mean ± SD) is the average performance of 5 individuals (- denotes decrease in weight).

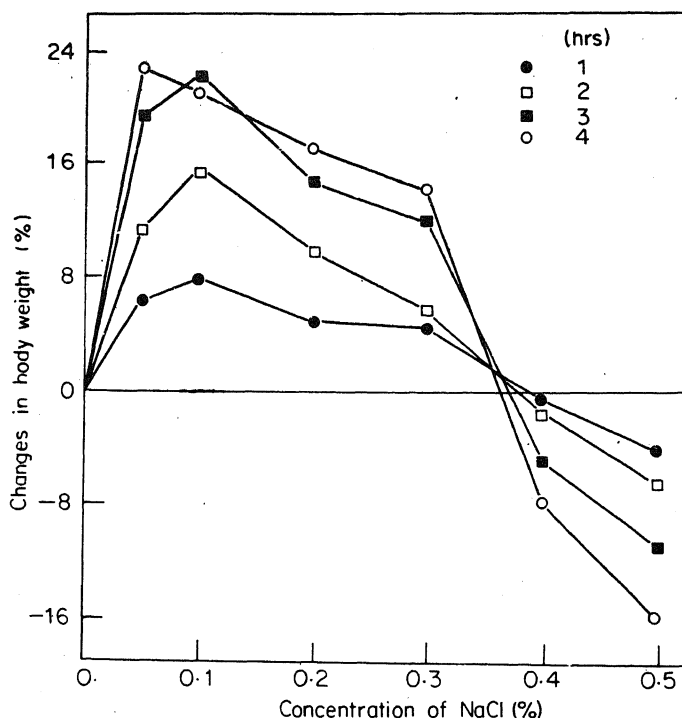


Figure 3. Changes in the body weight of *P. corethrurus* as a function of NaCl concentrations. Each value (mean) is the average performance of five individuals.

immersion in tap water. The weight difference between these two species may be due to the distribution in two different climatic zones and absorption of water through the body surface to hydrate the body tissues fully. Similar findings have been reported by Edwards and Lofty (1977).

The changes in body weight of *P. corethrurus* when immersed in different concentrations of sugar solutions are shown in table 3 and figure 2. Gradual increase in weight was found as a function of concentrations up to 4% sugar solutions. The weight increase, 21% in 1% sugar solution gradually increased to 29.3% in 4% at 4 hr immersion by absorption of water from the medium. In 5% sugar solution, loss of weight was noticed. The decrease in weight was gradual from 1 to 4 hr exposure due to loss of water from the body in response to external concentration of sugar medium. Graphical representation of these results (figure 3) indicates that the level of sugar solution at which *P. corethrurus* can control its body weight without loss or gain is 4.5%.

Changes in body weight of *P. corethrurus* were also observed as a function of NaCl concentrations and exposure time (table 4). *P. corethrurus* gained weight in concentrations up to 0.3%. But the gain in weight decreased as concentration increased from 0.05 to 0.3%. For instance, the gain in weight which was 22.8% of its body in 0.05% NaCl, decreased to 14.1% in 0.3% NaCl at 4 hr exposure. In higher concentrations, loss in its weight was observed. The loss in weight was 7.8% and 15.8% of its body weight in 0.4 and 0.5% NaCl respectively at 4 hr exposure. The weight increase in lower concentrations of NaCl might be due to absorption of water and the decrease in weight in higher concentrations might be due to loss of water from the body in accordance with external concentration. As visualized from figure 3, the salinity level at which *P. corethrurus* can regulate its

body weight without loss or gain is 0.375%. This is the survival limit. Hanumante and Nagabhushanam (1977) reported that the survival limit of NaCl for *P. excavatus* was 0.22%. This indicates that *P. corethrurus* is more tolerant to NaCl than *P. excavatus*.

The tolerance limit of sugar medium for *P. corethrurus* is generally greater than that of NaCl with reference to survival as well as weight regulation. This can be attributed to the different chemical nature of the substances. Only further studies on this line can throw more light on this aspect.

The weight changes in *P. corethrurus* when exposed to tapwater, sugar and NaCl concentrations reveal that it is a poor osmoregulator like other tropical species of earthworms (e.g. *Phretima posthuma*: Bahl 1945; *Perionyx excavatus*: Hanumante and Nagabhushanam 1977). This ability to change its body weight in accordance with the concentration of the substance is an ecophysiological adaptation of this species to overcome the adverse effects.

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Cooperative behaviour in rhesus monkeys living in urban and forest areas

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Abstract. Two urban and two forest groups of rhesus monkeys (*Macaca mulatta*) were observed for their behaviours like social grooming, social play, coalition relations and consort relations. Adult females played a major role in grooming all other age-sex classes. The grooming between adult males and females increased during mating season. Social play was confined to infants and juveniles. Young adult males participated in play during non-mating season. Adults of both sexes participated in coalition and consort relations.

Keywords. Social grooming; social play; coalition relations; consort relations; rhesus monkey; *Macaca mulatta*.

1. Introduction

Rhesus monkeys seek help from one another for food, movement, and to attack and defend themselves from predators (Pirta 1982). These exigencies could lead to the genesis of cooperative behaviour patterns. The hypothesis that hunting worked as a selective force in the evolution of cooperative life (Schaller 1972) may only be partially true (Teleki 1973). On the other hand, the fear of being hunted should have certainly favoured some kind of group life (Thorpe 1974). The coherence of a group requires development of strong interpersonal attachments or affectional bonds (Harlow and Harlow 1965). Observations on four behaviour patterns, which seem essential in the development of these affectional bonds, are reported here.

2. Methods

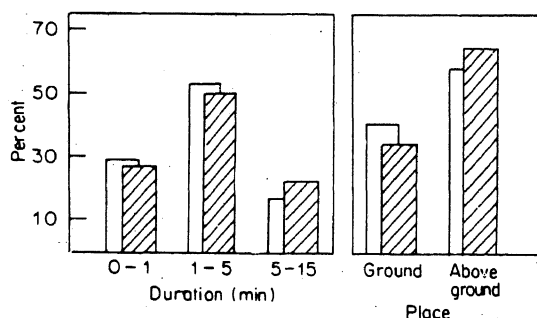
The details of study areas, groups observed, definitions of behaviour units, and methods of recording and analysis are given elsewhere (Pirta 1981). In brief, two forest groups, comprising 27 and 38 individuals, and two urban (temple) groups comprising 68 and 129 individuals, were observed from August 1977 to July 1978. The study areas were, Chakia Forest and temples (urban) located in Varanasi district of Uttar Pradesh. A total of 425 and 465 observational hr were spent on the forest and urban monkeys respectively. The *ad libitum* and sequence sampling were employed to collect data. Four types of behaviours are discussed in this paper: (i) Social grooming. One individual solicits, or is stimulated to solicit by another individual, and the latter then starts discretely picking and spreading its fur. (ii) Social play. Two or more individuals come together and engage in mutual chasing, running, sham biting, etc. (iii) Coalition relations. An association between two or more members of the same or different age-sex classes, identified by such behaviours as maintenance of proximity, reciprocal following, mutual defence, etc. (iv) Consort relations. An association between adult male and adult

Table 1. Episodes of social grooming observed in forest (F) and urban (U) monkeys.

	Duration (min)			Type		Place	
	0-1	1-5	5-15	Complete	Incomplete	Ground	Above ground
Forest	35 (29.9)	62 (53)	20 (17.1)	117 (22.12)	412 (77.88)	220 (41.6)	309 (58.4)
Urban	93 (27.3)	173 (50.7)	75 (22)	341 (24)	1082 (76)	489 (34.4)	934 (65.6)

		Groomee				Total
Groomer		Male	Female	Juvenile	Infant	
Male	F	22(40.7)	30(55.6)	2(3.7)	0	54(10.2)
	U	41(22.4)	117(64.3)	15(8.2)	10(5.5)	183(12.9)
Female	F	41(11.3)	140(38.6)	133(36.6)	49(13.5)	363(68.6)
	U	203(20.7)	459(46.8)	214(21.8)	105(10.7)	981(68.9)
Juvenile	F	4(3.6)	86(78.2)	13(11.8)	7(6.4)	110(20.8)
	U	53(23.1)	135(59.0)	33(14.4)	8(3.5)	229(16.1)
Infant	F	0	0	2(100)	0	2(0.4)
	U	11(36.7)	18(60.0)	1(3.3)	0	30(2.1)
Total	F	67	256	150	56	529
	U	308	729	263	123	1423

Numerical values denote number of episodes and its percentage (in parenthesis) for all tables.

**Figure 1.** Social grooming in forest (empty bars) and urban (hatched bars) monkeys.

female, identified by such behaviours as maintenance of proximity, reciprocal following, mutual grooming, courtship, etc.

3. Observations

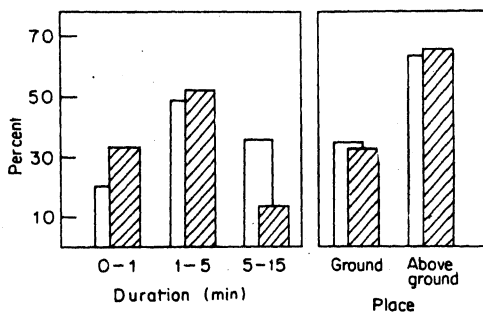
3.1 Social grooming

All age-sex classes participated in grooming. A total of 529 episodes in forest and 1423 episodes in urban monkeys were observed (table 1, figure 1). Sometimes

Table 2. Episodes of social grooming observed in urban monkeys during non-mating (NS) and mating season (MS).

		Duration (min)			Type		Place	
		0-1	1-5	5-15	Complete	Incom- plete	Ground	Above ground
Non-mating		59	89	23	171	715	297	589
season		(34.5)	(52.0)	(13.5)	(19.3)	(80.7)	(33.5)	(66.5)
Mating		34	84	52	170	367	192	343
season		(20.0)	(49.4)	(30.6)	(31.7)	(68.3)	(35.8)	(64.2)

Groomer		Groomee				Total
		Male	Female	Juvenile	Infant	
Male	NS	33(24.6)	79(59.0)	12(9.0)	10(7.4)	134(15.1)
	MS	8(16.3)	38(77.6)	3(6.1)	0	49(9.1)
Female	NS	107(17.9)	284(47.6)	124(20.8)	82(13.7)	597(67.4)
	MS	96(25.0)	175(45.6)	90(23.4)	23(6.0)	384(71.5)
Juvenile	NS	33(25.4)	71(54.6)	23(17.7)	3(2.3)	130(14.7)
	MS	20(20.2)	64(64.6)	10(10.1)	5(5.1)	99(18.4)
Infant	NS	11(44.0)	14(56.0)	0	0	25(2.8)
	MS	0	4(80.0)	1(20.0)	0	5(0.9)
Total	NS	184	448	159	95	886
	MS	124	281	104	28	537

**Figure 2.** Social grooming in urban monkeys during mating season (empty bars) and non-mating season (hatched bars).

grooming was reciprocal. In all, 214 episodes of simultaneous reciprocation, and 126 episodes of delayed reciprocation, were observed among the identified individuals of both habitats.

There were differences in grooming during mating (November to February) and non-mating (March to July) seasons. This analysis was done on urban monkey data only. The percentage and duration of male-female grooming episodes were greater during the mating season than during the non-mating season. The male-male, male-juvenile, and male-infant grooming increased during non-mating season while the male-female grooming decreased (table 2, figure 2).

Table 3. Episodes of social play observed in forest and urban monkeys.

	Duration (min)			Type		Place		
	0-5	5-10	10+	Complete	Incomplete	Ground	Tree	Mixed
Forest	4 (50)	4 (50)	0	8 (8.1)	91 (91.9)	12 (12.1)	81 (81.6)	6 (6.1)
Urban	19 (67.9)	5 (17.9)	4 (14.3)	28 (5)	535 (95)	280 (49.7)	220 (39)	63 (11.2)

	Participants*				Number		
	I-I	J-J	J-I	J-I-Y	2-5	5-10	10+
Forest	25 (25.3)	48 (48.5)	25 (25.3)	3 (3)	85 (85.9)	14 (14.1)	0
Urban	138 (24.5)	202 (35.9)	166 (29.5)	73 (13)	383 (68)	149 (26.5)	31 (5.5)

* I-I=infant-infant; J-J=juvenile-juvenile; J-I=juvenile-infant; J-I-Y=juvenile-infant-young adult males

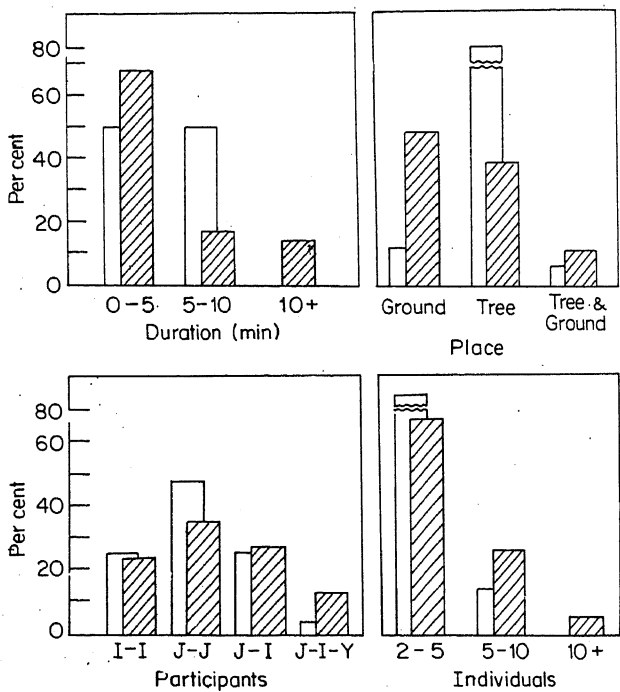


Figure 3. Social play in forest (empty bars) and urban (hatched bars) monkeys. Participants during social play include: I=infants; J=juveniles; Y=young adult males. (The broken bar indicates that the actual length of the bar has been reduced), bars indicate percent.

Table 4. Episodes of coalition relations observed in forest and urban monkeys.

Behaviours		Forest	Urban
Contact maintenance	Grooming	11 (13.6)	26 (43.3)
	Following	18 (22.2)	6 (10)
	Proximity	7 (8.6)	12 (20)
	Contact	5 (6.2)	10 (16.7)
	Searching	0	0
Cooperative attack	Male	9 (20.9)	23 (56)
	Female	23 (53.5)	9 (22)
	Others	11 (25.6)	9 (22)
Communal attack		34 (42)	3 (5)
Communal defence		17 (21)	0
Submissive mount		9 (11.1)	3 (5)

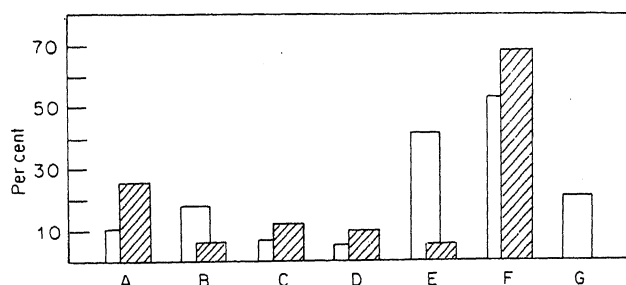


Figure 4. Coalition relations in forest (empty bars) and urban (hatched bars) monkeys. A = social grooming; B = following; C = proximity; D = contact; E = communal attack; F = cooperative attack; G = communal defence.

3.2 Social play

Infants, juveniles and young adult males (only in non-mating season) participated in social play while adult males and females did not. This activity included social exploration and manipulation, chasing, pulling, sham biting, claspings, hanging, vocalising, wrestling, aggressive posturing, swimming, snatching objects, etc. A total of 99 and 563 episodes of social play were observed in the forest and urban areas respectively (table 3, figure 3).

3.3 Coalition relations

Coalitions or alliances were observed within and between adult males and females. These relationships were multiplex or involved several types of interactions. The partners (mostly two monkeys) played both complementary and reciprocal roles. For analysing the characteristics of coalition relationship, each interaction between a pair or trio was counted as a separate episode. In view of limited data from Chakia Forest, it was supplemented by results from Asarori Forest monkeys. An affectionate relationship by the coalition partners was maintained by such behaviours as grooming, following, proximity and contact. The members of a pair showed cooperative attack and defence to meet the threatening situations. In all 81 and 60 episodes were analysed respectively from forest and urban groups (table 4, figure 4).

Table 5. Episodes of consort relations observed in forest and urban monkeys.

Behaviours		Forest	Urban
Contact maintenance	Grooming	53 (55.8)	113 (42.6)
	Following	72 (75.8)	207 (78.1)
	Proximity	61 (64.2)	137 (51.7)
	Contact	35 (36.8)	88 (33.2)
	Searching	5 (5.3)	9 (3.4)
Cooperative attack	Rival male	6 (40)	14 (30.4)
	Rival female	2 (13.3)	17 (37)
	Others	7 (46.7)	15 (32.6)
Cooperative defence	Rival male	6 (50)	45 (81.8)
	Rival female	3 (25)	6 (10.9)
	Others	3 (25)	4 (7.3)
Courtship		36 (37.9)	71 (26.8)

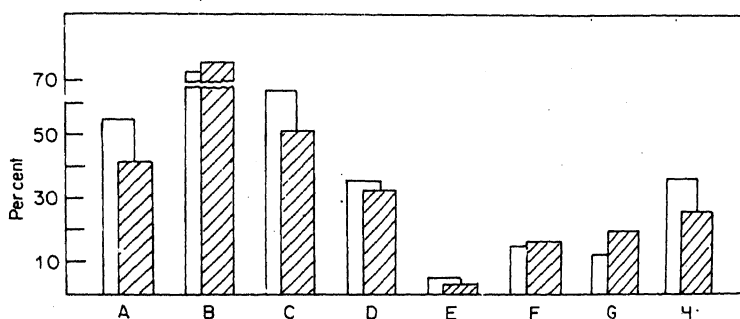


Figure 5. Consort relations in forest (empty bars) and urban (hatched bars) monkeys. A=social grooming; B=following; C=proximity; D=contact; E=searching; F=cooperative attack; G=cooperative defence; H=courtship. (The broken bar indicates that the actual length of the bar has been reduced).

3.4 Consort relations

Pair formation among adult males and adult females was observed during mating season (November to February). These relationships, termed as consort relationships, were multiplex or involved several types of interaction. Both partners of a pair behaved differently (or played a complementary role). The females showed specific preferences for male partners; however, the dominant males made consort relations with most of the females. A female made consort relations several times during the mating season. Each interaction between a consort pair was considered as a separate episode. A consort relationship was determined by such behaviours as grooming, following, proximity, contact, searching, cooperative attack and defence and finally, courtship. In all 95 and 265 episodes were analysed from forest and urban areas respectively (table 5, figure 5).

4. Discussion

The behaviour patterns described above help in the development of stable interpersonal relationships (or affectional bonds) which in turn give rise to a cohes-

sive group. A review of social grooming in Old World monkeys (Goosen 1980) suggests its role as a communicator of affection. Oppenheimer (1979) reported that grooming index seems to be the best indicator of the group's social structure. Social play in infancy and juvenile stages helps in the development of normal adult social relationships (Dolhinow and Bishop 1970; Suomi and Harlow 1971). Almost all cooperative activities seen in the adults were exhibited and practised during play. Bekoff (1978) proposes that social play has evolved primarily as a cooperative venture. Some members of the group form coalitions which help in development of peaceful social environment by monitoring the social field. Coalitions have been reported in several primate species (Boehm 1981). Adults of opposite sexes form short term affectionate relationships (or consortships) during mating season (Lindburg 1971). Consorting appears to be the optimal strategy of reproduction (Tutin 1979).

From this brief discussion it is clear that social grooming, social play, coalition relationship and consort relationship help in the development of cooperative group life in rhesus monkeys.

Acknowledgements

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Histology and histochemistry of the tegumental glands in *Ligia exotica* Roux (Crustacea: Isopoda)

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Abstract. The tegumental glands in *Ligia exotica* are located around the oesophagus and also in the tissues of maxillae and maxillipeds. Each gland consists of a number of acini and each acinus is made of a rosette of 8-11 large conical cells. Histochemical observations of these glands reveal the presence of strongly sulphated and carboxylated acid mucopolysaccharides and neutral mucopolysaccharides.

Keywords. Histology; histochemistry; tegumental glands; crustacea; *Ligia exotica*.

1. Introduction

The tegumental glands in Crustacea located in the tissues around the oesophagus and those nearer the tegument are histologically more or less similar. Based on their distribution and functions, they were known as 'salivary glands' (Hewitt 1907), 'labial glands' (Yonge 1924), 'oesophageal glands' (Erribabu *et al* 1979) and 'cephalic mucous glands' (Shyamasundari and Hanumantha Rao 1977). Various functions were attributed to the tegumental glands. Yonge (1924, 1932) described the detailed structure and functions of the tegumental glands in *Nephrops norvegicus* and *Homarus vulgaris* respectively. Gorvett (1946) studied the structure and functions of various kinds of tegumental glands in some isopods. The functional aspects of the tegumental glands have been elucidated by Pugh (1962) in *Uca pugnax*, *U. minax* and *U. pugilator*. Stevenson (1964) suggested that their development could be correlated with the moulting cycle.

Studies on tegumental glands of isopods have received meagre attention. Patane (1938, 1951), Radu and Cihodaru-Gheorghiu (1942) and Gorvett (1946, 1952, 1956) studied the histology of these glands in terrestrial isopods. Histological details of these glands in other crustaceans have been observed by Reddy (1937) in the crab *Paratelphusa hydrodromus*, by Pike (1947) in *Galathea*, by Barker and Gibson (1977) in *Homarus gammarus* and by Shyamasundari and Hanumantha Rao (1977) in amphipods.

Stevenson and Murphy (1967) have given a histochemical approach to the tegumental glands in the isopod *Armadillidium vulgare*. Barker and Gibson (1977) also studied the histochemical nature of these glands in *H. gammarus*. Shyamasundari (1979) and Erribabu *et al* (1979) elucidated the histochemistry of these glands in the amphipod *Talorchestia martensii* and the crab *Menippe rumphii* respectively. The present study attempts to elucidate the histochemical nature of the tegumental glands in *Ligia exotica*.

2. Material and methods

Specimens of *L. exotica* were collected from among and under the rocks submerged near the outer harbour of Visakhapatnam and Gangavaram. The head portions were dissected and fixed in Bouin's, Susa, formol calcium and Carnoy for 24 hr. Paraffin sections (10 μ m thickness) were stained in Heidenhain's Azan and Delafield's iron hematoxylin.

For histochemical studies the following methods mainly from Pearse (1968) and Bancroft (1975) were employed. Periodic acid / Schiff (PAS) technique for carbohydrates, PAS after saliva digestion for glycogen, PAS after acetylation and deacetylation for 1:2 glycol groups were employed.

Alcian blue (AB) technique at pH 2.5 and 1 for acid mucopolysaccharides was employed. Acid mucosubstances were distinguished from neutral mucosubstances by the combined technique of alcian blue at pH 2.5 and pH 1 / PAS.

To distinguish sulphomucins from sialomucins aldehyde fuchsin (AF), AF / AB (pH 2.5), AB / safranin techniques were employed. The following chemical blocking techniques were employed to confirm sialomucins and sulfomucins: mild methylation followed by AB staining at pH 2.5, active methylation-AB at pH 2.5, mild methylation-saponification / AB pH 2.5 and active methylation-saponification / AB pH 2.5. Toluidine blue method was used to identify the metachromatic nature of these cells.

Proteins were identified using the following techniques: Bromophenol blue method for basic proteins, Millon's reaction for tyrosine, *p*-dimethylamino-benzaldehyde-nitrite (*p*-DMAB--nitrite) method for tryptophan, KMnO_4 / AB method for disulphide groups, ferric ferricyanide technique for sulphhydryl groups, ninhydrin / Schiff method for protein-bound amino groups and Congo red technique to demonstrate glycoproteins.

To identify the lipids, the techniques of Sudan black B (for saturated lipids) and copper phthalocyanin (for phospholipids) were employed.

The nucleic acids were demonstrated by employing Feulgen reaction for DNA and pyronin Y method for RNA.

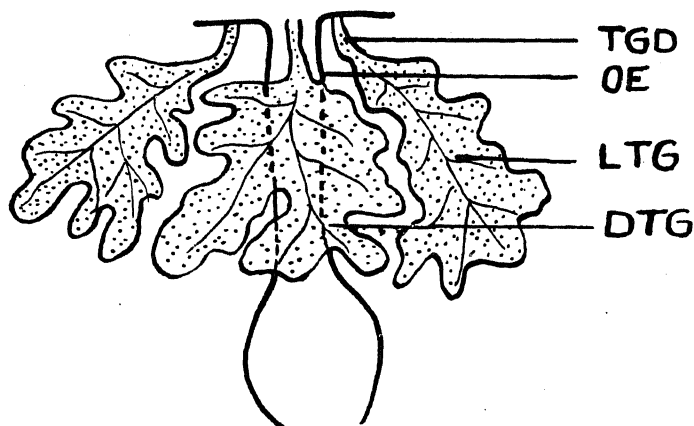
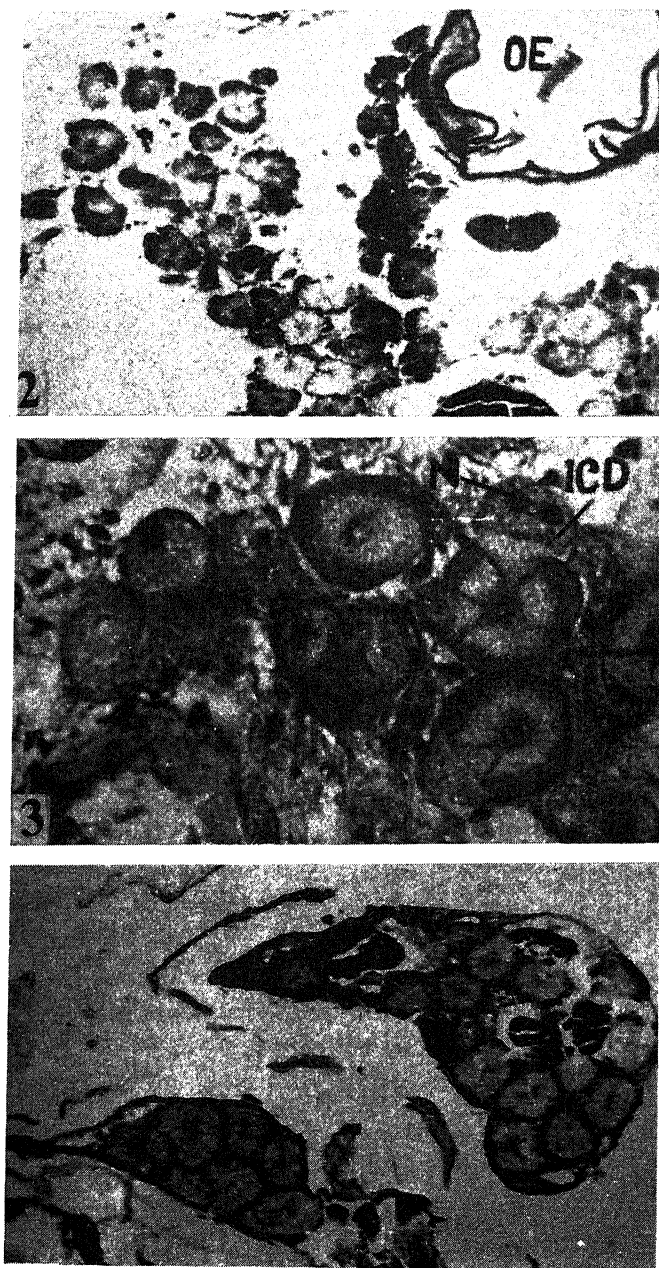


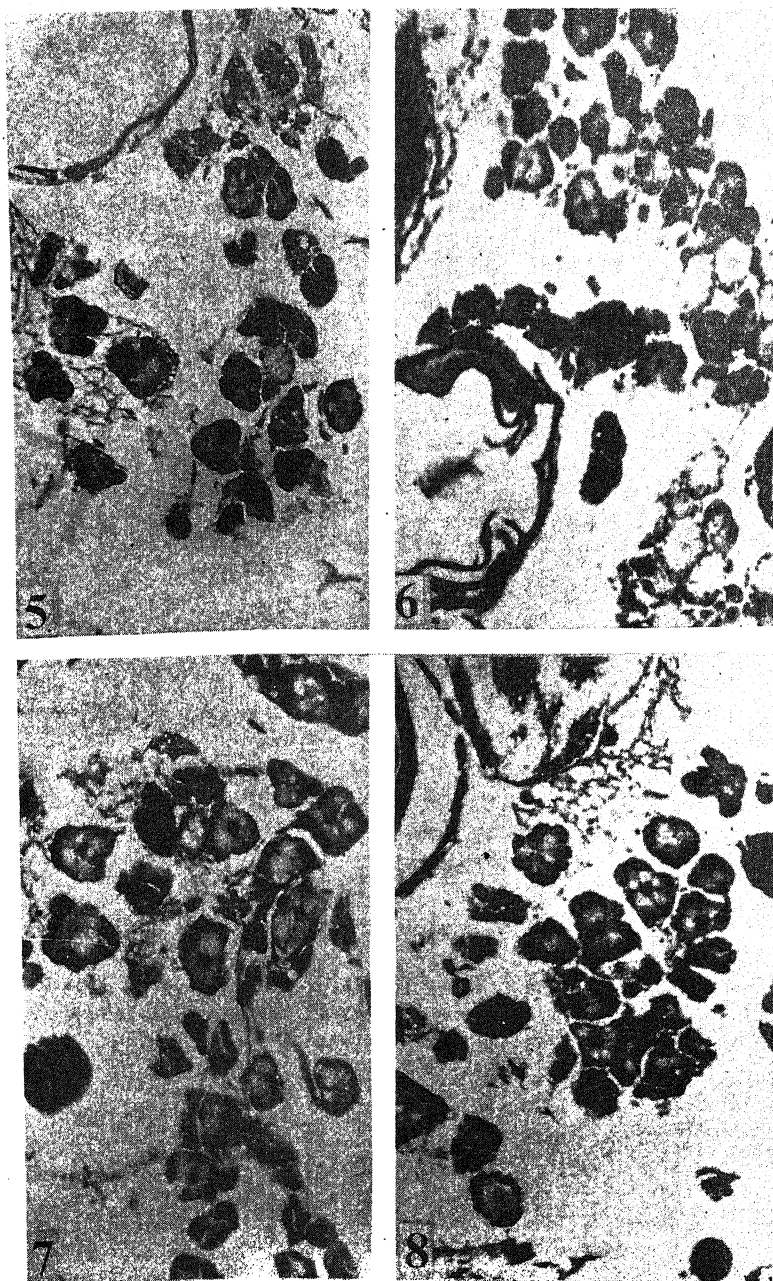
Figure 1. Diagrammatic representation of tegumental glands showing their ducts. DTG - Dorsal group of tegumental glands; LTG - Lateral group of tegumental glands; OE - Oesophagus; TGD - tegumental gland duct.



Figures 2-4. 2. Transverse section (TS) through cephalic region showing tegumental glands surrounding the oesophagus (OE) (Azan) $\times 50$ 3. TS showing enlarged view of tegumental glands with intracellular ducts (Azan) $\times 250$. ICD-Intracellular duct; N - Nucleus. 4. TS showing the maxillipede group of tegumental glands (Azan) $\times 50$.

3. Observations

The tegumental glands of *L. exotica* are located in the cephalic region around the oesophagus. One group of glands is situated above the oesophagus at the base of the labrum and a pair of glands is distributed on either side of the oesophagus. The



Figures 5-8 $\times 60$. 5. TS of tegumental glands stained with periodic acid / Schiff (PAS). 6. TS of tegumental glands stained with alcian blue (AB) at pH 2.5 showing acid mucopolysaccharides. 7. TS of tegumental glands stained with AB at pH 2.5/ PAS showing acid and neutral mucopolysaccharides. 8. TS of tegumental glands positive to copper phthalocyanin showing large quantities of phospholipids.

oesophageal group of glands is larger than the other groups of glands and flank the ventro-lateral sides of the oesophagus. The ducts of these glands are directed towards the margin of the labium and open at the tip of the labrum and the mouth

parts (figure 1). Similar glands are also observed in the tissues of maxillae and maxillipeds.

3.1 Histology

The transverse section of each group of tegumental glands appears to be flattened. Each gland consists of a number of spherical acini and each is composed of a rosette of 8-11 large conical cells (figure 2). The entire gland is surrounded by a delicate sheath of connective tissue with small nuclei which can sometimes be seen between the gland cells. Each gland cell has a broad base and a narrow apex pointed towards the inside, thus forming a cavity in the central region of the acinus. Each cell is 30 μm in diameter at its broadest end and 42 μm in length. Two zones could be noticed in the cytoplasm of these cells. The peripheral cytoplasm is more granular with a large, oval and basal nucleus, while the central core is less granular. The nucleus measures 2 μm in diameter and each has a conspicuous nucleolus. A fine duct, oval in shape with a nuclei at its apex arises from the central cavity of each acinus to release the gland secretions (figure 3). These glands are also found in the tissues of maxillae and maxillipeds having the same histological plan (figure 4).

3.2 Histochemistry

A series of histochemical techniques has been applied to the sections of the tegumental glands to unravel the nature of substances present in the gland cells.

With the standard PAS technique (figure 5) a positive reaction was observed, which was resistant to saliva digestion showing the absence of glycogen. This reaction was however abolished by acetylation and restored after deacetylation indicating the presence of 1:2 glycol groups.

The gland cells show a positive response to AB at both pH 2.5 and 1 (figure 6) indicating the presence of both sulphated and carboxylated mucopolysaccharides. When a combined technique of AB pH 2.5/PAS was employed, the central part of the gland cells stained blue and the peripheral region stained magenta, suggesting the presence of acid mucopolysaccharides in the former and neutral mucopolysaccharides in the latter. With a combined AB pH 1/PAS technique (figure 7), the central cytoplasm of the gland cells stained blue and peripheral cytoplasm showed magenta indicating the presence of sulphated acid mucopolysaccharides, sialomucins in the former and neutral mucopolysaccharides in the latter.

With aldehyde fuchsin (AF) and AF/AB techniques they stained purple indicating the presence of sulphated mucosubstances. In the combined technique of AB with safranin, the gland cells stained orange suggesting the presence of strongly acid mucosubstances.

The presence of sialomucins and sulphomucins was confirmed by the following reactions. The alcianophilia was not abolished after mild methylation indicating the presence of sulphated mucosubstances. The blue colour was removed completely after active methylation suggesting the presence of sulphated and carboxylated mucosubstances. The alcianophilia was retained after saponification indicating the presence of carboxylated mucosubstances in the gland cells. With toluidine blue the glands exhibited metachromasia.

The gland cells showed a positive response to mercury bromophenol blue for basic proteins but reacted negatively to Millon's reaction and *P*-DMAB - nitrite for tyrosine and tryptophan. They gave a moderate reaction to KMnO_4 /AB method for disulphide groups. With ferric ferricyanide technique the gland cells showed a

Table 1. Histochemical tests applied to the tegumental glands

Histochemical test applied	Result
Periodic acid / Schiff (PAS)	++
PAS / saliva	++
Acetylation / PAS	-
Deacetylation / PAS	++
Alcian blue (AB) pH 2.5	+
AB pH 1	+
AB pH 2.5 / PAS	Central cytoplasm stained blue Peripheral cytoplasm stained red
AB pH 1 / PAS	
Aldehyde fuchsin (AF)	+++
AF / AB	Purple
AB / safranin	Orange
Mild methylation / AB	++
Mild methylation-saponification / AB	++
Active methylation / AB	-
Active methylation-saponification / AB	++
Toluidine blue	Metachromasia
Bromophenol blue	+
Millon's reaction	-
p-DMAB-nitrite	-
KMnO ₄ / AB	+
Ferric ferricyanide	++
Ninhydrin / Schiff	+
Congo red	+
Sudan black B	++
Copper phthalocyanin	++
Feulgen reaction	++
Pyronin Y	++

+++ = Intensely positive; ++ = strongly positive; + = moderately positive; - = negative.

strong reaction for sulphhydryl groups. They exhibited moderate positivity to ninhydrin / Schiff and Congo red techniques for protein bound amino groups and glycoproteins respectively.

A strong reaction to Sudan black B and copper phthalocyanin techniques was shown (figure 8) demonstrating the presence of lipids and phospholipids. With Feulgen reaction and pyronin Y technique the gland cells showed a positive response due to the presence of DNA and RNA. Results of these histochemical tests are summarised in table 1.

4. Discussion

The tegumental glands in *L. exotica* are embedded in the surrounding tissue of the oesophagus, an observation which is in agreement with that of Gorvett (1946). According to some workers (Yonge 1924; Reddy 1937; Pike 1947; Barker and Gibson 1977; Shyamasundari and Hanumantha Rao 1978; Erribabu *et al* 1979) such glands are embedded in the connective tissue of the oesophagus.

Stevenson (1964) observed that the intracellular duct of these glands possesses nuclei in *A. vulgare*. Yonge (1924) and Reddy (1937) reported that the cavity of each gland is a continuation of the intracellular duct. Pugh (1962) states that the duct is a sort of a syncytium consisting of intracellular canals. The present observations agree with that of Stevenson (1964).

Several functions have been attributed to the tegumental glands of decapods and isopods. Ide (1892) stated that they function as salivary glands due to their restriction to the head region. Generally parasitic isopods have poorly developed digestive systems. Hence, Ide (1892) considered that the glands are well developed in parasitic species than in free living forms. Murlin (1902) observed that the secretions of hepatopancreas are involved in digestion and hence there is no need for salivary secretion in isopods. Yonge (1924, 1932) stated that they have no digestive role, but the secretions play an important role in the formation of chitinous lining in the foregut and hindgut in *N. norvegicus*. Reddy (1937) supported this view and stated that the secretions of the tegumental glands helped in the entanglement of food and in the formation of the chitin. Gorvett (1946) concluded that the rosette glands of land isopods take part in the formation of the epicuticle and also the thickening and sclerotization of the tips of the mouth parts. Pugh (1962) in his studies on *Uca pugnax* pointed out that these glands secrete a lubricating substance, which aids in binding the faecal matter into masses. Similar observations were made by Shyamasundari and Hanumantha Rao (1978) in *Thenus orientalis*. Stevenson (1964) observed that the development of tegumental glands in *A. vulgare* could be correlated with moulting cycle. Schmitz (1967) also expressed a similar opinion from his observations on *Gammarus lacustris lacustris*. According to Erribabu *et al* (1979) the secretions play a role in the free passage of the food material and also partly aid in digestion.

The gland secretions in *L. exotica* are mostly strongly sulphated and carboxylated acid mucopolysaccharides including sialic acid and hyaluronic acid and also neutral mucopolysaccharides. A small amount of glycoproteins and lipids was also observed. Stevenson and Murphy (1967) reported that the secretions of the tegumental glands in *A. vulgare* are acid mucopolysaccharides and carbohydrate protein in nature. No glycogen was present in these glands. According to Barker and Gibson (1977) these glands secrete acid mucopolysaccharides, acid phosphatase and ATPase in *H. gammarus*. The gland secretions in *T. martensii* are acid and neutral mucopolysaccharides along with glycoproteins (Shyamasundari 1979), a finding which agrees with the present observations. From the ensemble of the foregoing results it can be clearly stated that the mucous nature of the gland secretions aids in swallowing the food material. The gland secretions may also play a role in digestion due to their acidic nature.

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Influence of hypoxia on metabolism and activity in *Puntius sarana* (Hamilton) (Pisces: Cyprinidae)

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Abstract. In *Puntius sarana*, changes in metabolic rates in relation to ambient oxygen fell into the general pattern at 30 and 35 °C, the differences being mainly in the levels of metabolism, both aerobic and anaerobic. The mean RQs at high ambient oxygen were 0.77 and 0.63 revealing that the fish were aerobic under adequate ambient oxygen. The hypoxic RQ were 1.42 and 1.90 suggesting that the fish derived considerable energy anaerobically. The aerobic AQs ranged between 0.06 and 0.18 depending on the utilization of protein and the hypoxic AQs were in consonance with the corresponding changes in hypoxic RQs. As judged from the asphyxial oxygen level, *P. sarana* can live, below air saturation, up to 0.41 and 0.49 mg O₂/l at 30° and 35°C respectively. The random activity of the fish increased with decrease of ambient oxygen.

Keywords. Metabolism; respiratory quotient; ammonia quotient; random activity; ambient oxygen; hypoxia; *Puntius sarana*.

1. Introduction

The multiplicity of involvement between environment and metabolic rate illustrates that within the bounds of knowledge the factors of dissolved oxygen, temperature and activity exert the greatest effect on metabolism — in unpolluted waters. *Puntius sarana* (Hamilton) is one of the economically important carps not subjected to proper experimental study which describes the effects of dissolved oxygen and temperature. This paper deals with the influence of hypoxia on metabolic rates and quotients (RQ and AQ[†] and random (spontaneous) activity in this species. Increase in RQ over unity was demonstrated in goldfish and rainbow trout (Kutty 1968), *Tilapia mossambica* (Kutty *et al* 1971a; Peer Mohamed and Kutty 1981) and in *Rhinomugil corsula* (Kutty and Peer Mohamed 1975) exposed to low ambient oxygen. To determine whether the ammonia produced is of use to freshwater fish under anaerobiosis (Peer Mohamed and Kutty 1981), relative changes in ammonia excretion and RQ have been estimated to study the utilization of proteins by fish.

† Respiratory quotient, RQ = volume of CO₂ produced / volume of O₂ consumed; ammonia quotient, AQ = the volume or mole: mole relation of NH₃-N excreted to O₂ consumed as explained in Kutty (1972)

2. Material and methods

Minor carp, *Puntius sarana* were collected from the Vaigai Dam (Tamil Nadu, India) and kept in a tank (300 litre) for some days and then transferred to glass aquaria (70 litre) for acclimation. Experimental fish was deprived of food for 36 hr (Peer Mohamed and Kutty 1981) and left in the respirometer overnight before experiments. Nine fish ranging in total length from 16.4 to 17.1 cm (mean 16.7 cm) and in weight from 26.6 to 27.9 g (mean 27.1 g) were tested at $30 \pm 0.5^\circ\text{C}$ and $35 \pm 0.5^\circ\text{C}$.

The apparatus used was a modification of Fry's respirometer (Kutty *et al* 1971b) in which metabolic rates and random activity can be simultaneously measured. The annular transparent perspex respirometer (3 litre) was designed such that the diffusion of gases into and out of the water in the respirometer was minimised. Decarbonated tap-water, adjusted to a pH of 8.2 (Kutty 1968, 1972; Kutty *et al* 1971a), was used for experiments as explained in Kutty *et al* (1971a). From an overhead glass tank (70 litre) water flowed through the respirometer to a ground level tank of the same capacity from which it was pumped for recirculation.

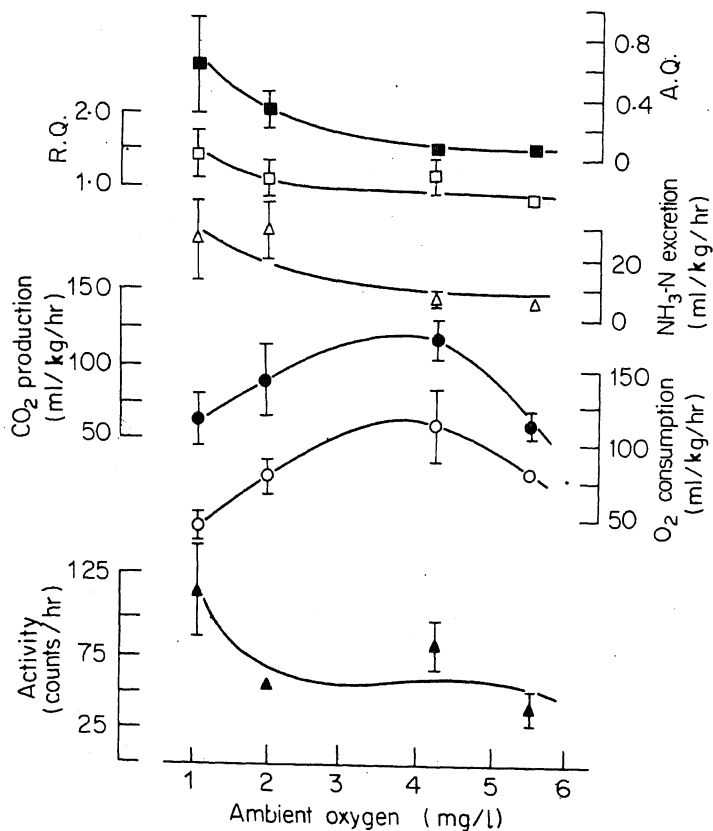


Figure 1. Oxygen consumption, carbondioxide production, ammonia excretion, RQ, AQ and random activity in relation to ambient oxygen below air saturation in the minor carp, *Puntius sarana* acclimated to and tested at 30°C . Each value plotted is a mean of ($\pm$ S.E.) 4 determinations. The SE is not indicated if it falls within the area of symbol shown in the figure.

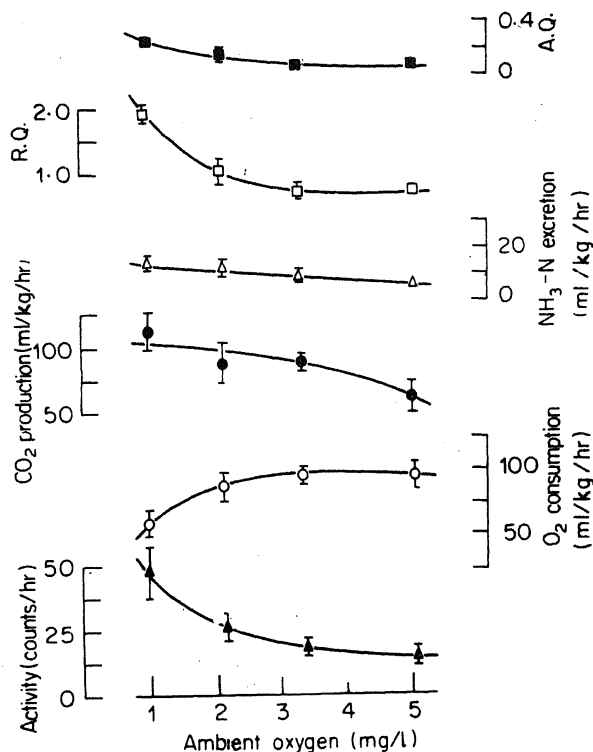


Figure 2. Oxygen consumption, carbon dioxide production, ammonia excretion, RQ, AQ and random activity in relation to ambient oxygen in *Puntius sarana* acclimated to and tested at 35°C. See legend to figure 1 for further explanation.

The experimental procedure followed was as described in Kutty and Peer Mohamed (1975) with closed and open periods which together constitute a run. Each experiment consisted of 5-8 runs of 1 hr in duration when the respirometer remained closed. The ambient oxygen fell from air saturation to the asphyxial level (the oxygen concentration at which the fish begin to lose equilibrium) due to the depletion of oxygen by the respiration of the fish itself. Then the respirometer was flushed with air-saturated water to revive the animal.

2.1 Methods of water analyses

Dissolved oxygen was measured using unmodified Winkler technique (American Public Health Association 1965). The size of the sample for titration was 25 ml. Maros-Schulek technique (Maros *et al* 1961) modified for fish metabolism studies by Kutty *et al* (1971a) was followed for total carbondioxide estimation. The method of Stroganov (1962) as described by Kutty (1972) was followed for ammonia measurement. The size of the sample was 50 ml.

3. Results

The trend of oxygen consumption, carbondioxide production, ammonia excretion, RQ, AQ and random activity below air saturation in *P. sarana* acclimated to and tested at 30 and 35°C are graphically shown in figures 1 and 2 respectively. In table 1 are presented for comparison the mean values of metabolic rates, quotients and random activity at high (normoxic) and low (hypoxic) oxygen concentrations tested.

Table 1. Metabolic rates, RQ, AQ and random activity in *Puntius sarana* in high and low oxygen at 30 and 35°C.

	30°C		35°C	
	High O ₂	Low O ₂	High O ₂	Low O ₂
Mean ambient O ₂ —mg/l	5.45 ± 0.08	1.02 ± 0.10	5.14 ± 0.10	0.99 ± 0.30
Mean rate of O ₂ consumption—ml/kg/hr	78.50 ± 1.50	42.3 ± 8.7	94.8 ± 8.6	61.0 ± 9.4
Mean rate of CO ₂ production—ml/kg/hr	60.3 ± 6.9	63.0 ± 17.4	61.0 ± 10.9	113.0 ± 14.0
Mean rate of NH ₃ -N excretion—ml/kg/hr	4.7 ± 0.4	25.7 ± 13.6	5.4 ± 0.6	15.6 ± 2.7
Mean respiratory quotient—RQ	0.77 ± 0.10	1.42 ± 0.31	0.63 ± 0.05	1.90 ± 0.13
Mean ammonia quotient—AQ	0.060 ± 0.006	0.626 ± 0.330	0.056 ± 0.007	0.256 ± 0.020
Mean random activity counts/hr	41.0 ± 17.5	117.0 ± 32.0	27.6 ± 1.5	57.5 ± 11.8

The 'high' oxygen refers to a mean ambient oxygen concentration near air saturation and the 'low' oxygen refers to the lowest mean ambient oxygen concentration tested, i.e. the mean of initial and final oxygen value of a run, the final value of which is the asphyxial oxygen concentration. Each value is ± S.E. of four determinations.

The trend lines of oxygen consumption at both temperatures are similar at oxygen levels between 4 and 1 mg/l. At 30°C the oxygen consumption at the high ambient oxygen level tested (near air saturation) was low (80 ml/kg/hr), the lowest rate was 41 ml/kg/hr at 0.79 mg O₂/l and the highest was 178 ml/kg/hr at 3.9 mg O₂/l. At 35°C the oxygen consumption decreased with decrease of ambient oxygen, 108 ml/kg/hr at 4.9 mg O₂/l and 43 ml/kg/hr at 0.74 mg O₂/l.

The trend lines of carbondioxide production at 30°C (figure 1) resembles that of oxygen consumption, in that there was a decrease in carbondioxide production with decrease in ambient oxygen below the middle range.

The trend in ammonia excretion indicates that ammonia excretion increased with decrease in ambient oxygen at 30°C; the maximum rate being 20 ml/kg/hr during the hypoxic phase (1 mg O₂/l), and the lowest level being 10 ml/kg/hr during normoxic phase (air saturation).

The routine RQ increased with decrease in ambient oxygen and RQ was near unity at high oxygen concentrations. The RQ shot up above unity during hypoxia and reached up to 1.5 (30°C) and 2 (35°C) at 1 mg O₂/l.

The trend lines of random activity at both temperatures are similar. At 30°C during high ambient oxygen the random activity almost doubled that of 35°C. The maximum random activity recorded were 117 and 58 counts/hr during hypoxic phase at 30 and 35°C respectively.

4. Discussion

The routine oxygen consumption at both temperatures followed almost the same general pattern, displaying low rates at high and low oxygen concentrations test-

ed (table 1) and high rates in the middle range. This agrees with the observations made earlier on goldfish and rainbow trout (Kutty 1968), *R. corsula* (Kutty and Peer Mohamed 1975) and *T. mossambica* (Peer Mohamed and Kutty 1981).

The RQs of *P. sarana* at high ambient oxygen are 0.77 at 30°C and 0.63 at 35°C (table 1) which are close to unity. It can, however, be pointed out that routine RQs of *Puntius* are closest to fat RQ values. The fish tested may have used more fat than carbohydrates and proteins. The tolerance of hypoxia can also be indicated, at least partially, by the asphyxial oxygen level, anaerobic abilities of the species under hypoxia. The asphyxial level of *P. sarana* was 0.41 and 0.49 mg O₂/l at 30 and 35°C respectively.

The routine RQ values at the low oxygen concentrations tested are significantly above unity (table 1) which suggest that during the hypoxic phase considerable anaerobic metabolism has taken place, releasing extra carbondioxide. The intensity of anaerobic metabolism was higher at 35°C than at 30°C. In this case it is known that the hypoxic RQs (table 1) are sustained for about 1 hr. Kutty (1968) showed that goldfish at 20°C sustained an RQ of about 2 for months at 1.5 mg O₂/l and found that their anaerobic abilities as indicated by the RQ did not increase. But, in the present study no experiments were done on fish acclimated to low oxygen, and therefore the full hypoxic abilities of the species are perhaps not known. However, it must be stated that the present tests were conducted in a closed system. An open system, with continuous flow of low oxygenated water, might indicate tolerance of oxygen lower than that presently tested.

The AQ values at high ambient oxygen staying around an average of 0.1, agree with that reported for *Gambusia* (Stroganov 1962) and also for *R. corsula* (Kutty and Peer Mohamed 1975). Under the hypoxic conditions (low ambient oxygen tested) the AQ values of *P. sarana* were 0.63 and 0.26 at 30 and 35°C respectively, which clearly show that AQs under hypoxia are higher than those for fish in high oxygen, in consonance with the changes of RQ under similar conditions. The temperature effect on the hypoxic AQs shows that the relative protein utilization would be less at 35°C, as indicated by the AQ (table 1).

The increased ammonia excretion under hypoxia resulting from anaerobic degradation of protein/aminoacids, but not in all cases, is thus indicated by the magnitude of the AQs as a possible maximum of 0.33 is suggested from the activity considerations (Kutty 1978). As referred to earlier, the AQ and RQ increased under hypoxia, suggesting a coupling of the increased ammonia excretion and increased carbondioxide output at low oxygenated environment (Kutty 1972). This may have a special significance in acid-base balance and also in conserving Na⁺ in freshwater fish (Kutty 1972; Kutty and Peer Mohamed 1975; Peer Mohamed and Kutty 1981). Hochachka and Somero (1973) suggested that anaerobic metabolism of carbohydrate and protein (aminoacids) can provide considerable amount of energy through pathways other than those for conventional glycolysis, such as those involved in the simultaneous breakdown of carbohydrates and aminoacids (Hochachka *et al* 1973). The high RQs and AQs in *P. sarana* may thus be of much significance in its survival.

P. sarana showed an increase in random activity on exposure to low oxygen (hypoxia), as described for several fish (Hamsa and Kutty 1972; Peer Mohamed and Kutty 1982). Increased activity induced by hypoxia might allow the fish to move out of the hypoxic environment to more oxygenated waters, if available. The increase of random activity to low ambient oxygen may have a major role

in the survival of *P. sarana* (Hamsa and Kutty 1972; Peer Mohamed and Kutty 1982).

The information obtained in the present study although provides some insight into the impact of low oxygen (hypoxia) on metabolism and activity, more information is necessary to elucidate the metabolic and behavioural responses of more species especially in view of their importance as cultivable warm-water fishes. However, the involvement of environmental interaction is not a deterrent to progress but a measure of the complexity to be met.

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Effect of steroid injection on food utilization in *Channa striatus*

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Abstract. Synthetic anabolic sex steroid hormones such as methyltestosterone (MT), testosterone (T), testosterone propionate (TP), estroid (ED), diethylstilbestrol (DES), diethylstilbestrol dipropionate (DES dipro) and docabolin (DN) were tested separately for their effect on food utilization in *Channa striatus*. Injection of low dosages of DES, DES dipro and TP acts as appetite depressors and Dn, MT, Ed and T as stimulators. MT, Ed and Dn induced not only increased feeding but also increased conversion. T promoted growth along with food consumption. Though DES and DES dipro suppressed feeding; individuals receiving these hormones showed increased growth with increasing dosage.

Keywords. Sex hormones; steroid injections, *Channa striatus*.

1. Introduction

The application of steroid sex hormones to enhance the rate and efficiency of food intake in animal husbandry resulted in substantial savings in production cost in United States (Monfort 1974). This finding has attracted the attention of aquaculturists, especially those producing highly esteemed but carnivorous fish. Unfortunately, the efficacy of several steroids on their positive growth effort is a matter of dispute, the experimental evidences being contradictory. For instance, DES, a steroid known for its high growth promoting ability in animal husbandry, is known to accelerate the growth rate and food conversion efficiency in the plaice *Pleuronectes platessa* (Cowey *et al* 1973). On the other hand, a number of workers have obtained contradictory results, indicating the growth depressing effect of DES (Bulkley 1972: *Ictalurus punctatus*; Matty and Cheema 1978: *Salmo gairdneri*).

A critical survey of literature reveals that to realize the growth-promoting ability, a suitable hormone and an appropriate dosage must be identified for the chosen species of fish; secondly, most of the previous workers have not attempted to experiment with an array of steroid hormones and a range of dosages to identify a suitable steroid; thirdly, previous studies have not identified whether the accelerated growth is due to enhanced appetite or improved food conversion efficiency or due to both. This paper reports the effect of a number of steroids on food consumption and conversion in the economically important air-breathing fish *Channa striatus* treated with different dosages of the selected hormones.

2. Material and methods

Juveniles of *C. striatus* (10 ± 2 g) were acclimated to laboratory conditions and feeding. For testing each hormone, five groups, each comprising a minimum of three individuals were used. The experiments were performed in round plastic

troughs (40 cm diameter, 14 l capacity) for 21 days. Room temperature averaged $28 \pm 2^\circ\text{C}$. The aquarium water was changed every 3 days. The dissolved oxygen content of the aquarium water was estimated following the unmodified Winkler's method (Welch 1948).

The fish were fed everyday at 0900 hrs on weighed live *Lepidocephalichthys thermalis* which was left in the test aquaria for a maximum period of 2 hr and the residual prey-remains collected subsequently with a hand sieve and dried to weight constancy at 80°C .

Faeces was collected and dried every three days by filtering the entire aquarium. The sacrifice method (Maynard and Loosli 1962) was followed to determine water and energy contents of the test individuals before the experiment. All the experimental individuals were starved for 24 hr to ensure complete evacuation of gut, and then weighed to an accuracy of 0.1 mg. Caloric contents of fish, food and faeces were determined using a 1412 semi-micro bomb calorimeter (Parr Instrument Co., Moline, USA).

The bioenergetics components were determined using the following formulae :

$$\text{Feeding rate (Cr)} = \frac{\text{Food consumed (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Food absorbed (cal/day)} = \text{Food consumed (cal/day)} - \text{Faeces egested (cal/day)}$$

$$\text{Absorption rate (Ar)} = \frac{\text{Food absorbed (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Absorption efficiency (\%)} = \frac{\text{Food absorbed (cal/day)}}{\text{Food consumed (cal/day)}} \times 100$$

$$\text{Conversion rate (Pr)} = \frac{\text{Growth (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Conversion efficiency (\%)} = \frac{\text{Growth (cal/day)}}{\text{Food consumed (cal/day)}} \times 100$$

$$\text{Metabolic rate (Mr)} = \text{Absorption rate} - \text{Conversion rate}$$

Details of the chosen hormones are given below :

Common name	Systematic nomenclature	Manufacturers
Androgenic		
Testosterone (T)	17 β - Hydroxy-androst-4-ene-3	German Remedies, India
Testosterone propionate (TP)	17 β - Acetoxy-androst-4-ene-3 one	German Remedies, India
17 α -Methyl-testosterone (MT)	17 β -Hydroxy-17 α -Methyl-androst-4-one-3-one	Sigma: St. Louis, USA
Estrogenic		
Diethylstilbesterol (DES)	4,4' (1,2-Diethyl-1, 2-ephenediyl bisphenol)	Sigma, St. Louis, USA

Diethylstilbesterol dipropionate (DES dipro)	Dipropionyl ester of x-x'-diethyl 4,4-stilbesterol diol	German Remedies, India
Docabolin (Dn)	17 β -(3-Phenylpropionyloxy oester-4 ene-3 one)	Organen (India Limited)
Estroid (Ed)	Dienesterol + Thyroid	Unichem Laboratories Limited

2.1 Hormone administration

The dosages of the chosen hormones administered were: 0, 5, 10, 20 and 30 mg/kg fish. The hormones were dissolved and diluted with sesame oil (carrier solution). Volume of the hormone solution injected into the fish was maintained constant at 60 μ l for all the tested dosages. DES was administered intramuscularly, intraperitoneally and orally through food. Nirmala (1981) found that intramuscular administration of the hormone enhanced the conversion efficiency of *C. striatus* to the highest level of 47% compared with 28 and 31% when administered intraperitoneally and through diet. Therefore, the chosen hormones were injected intramuscularly at the caudal region midway between the lateral line and ventral fin. Injections were given within a handling duration of 2 min without much disturbance to the individuals. Fish in the control group received sham injection of sesame oil alone. For injection, a 100 μ l capacity sterilised tuberculin syringe (Scientific Glass Engineering, Australia) was used.

The results obtained were subjected to statistical analysis such as student's *t* test and two-way analysis of variance.

3. Results

3.1 Feeding rate

The data presented in table 1 reveals that *C. striatus* receiving MT, Dn and T injections consumed more food than those in the respective control groups. Individuals receiving Ed (except those in the 10 mg/kg dosage group) also displayed a higher feeding rate than those in the control group. Whereas the feeding rate of the individuals receiving Dn progressively increased with dosage, the trend was not consistent in those treated with MT, T and Ed. In the T series, the lowest dosage of 5 mg/kg evoked the highest feeding rate of 96 cal/g/day; at higher dosages of 10, 20 or 30 mg/kg, the rate decreased to 82 and 77 cal/g/day. DES and DES dipro especially in lower dosages depressed food consumption. Considering

Table 1. Feeding rate (cal/g/day) of *Channa striatus* as a function of dosage of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	72 $\pm$ 2.9	72 $\pm$ 2.4	91 $\pm$ 2.1	78 $\pm$ 2.0	75 $\pm$ 2.2	71 $\pm$ 2.7	77 $\pm$ 2.7
5	80 $\pm$ 2.1	83 $\pm$ 2.2	110 $\pm$ 2.6	73 $\pm$ 2.4	65 $\pm$ 2.3	96 $\pm$ 2.4	67 $\pm$ 2.8
10	110 $\pm$ 1.6	69 $\pm$ 1.2	112 $\pm$ 2.3	66 $\pm$ 2.4	65 $\pm$ 2.3	82 $\pm$ 2.1	89 $\pm$ 2.1
20	92 $\pm$ 1.7	77 $\pm$ 2.5	118 $\pm$ 2.5	80 $\pm$ 2.8	64 $\pm$ 2.0	77 $\pm$ 2.0	56 $\pm$ 2.4
30	113 $\pm$ 1.4	82 $\pm$ 1.9	129 $\pm$ 2.8	77 $\pm$ 2.2	80 $\pm$ 2.1	77 $\pm$ 2.7	71 $\pm$ 0.2

Each value represents the mean ($\pm$ SD) performance of 3-6 individuals.

Table 2. Absorption rate (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	65±1.2	66±1.2	84±1.9	72±2.3	69±2.3	65±1.7	73±2.6
5	73±2.3	76±2.9	100±2.7	67±2.0	59±1.3	87±2.3	62±2.0
10	99±1.5	62±1.0	103±2.5	58±2.9	59±2.6	75±1.9	82±2.8
20	83±2.8	71±2.6	103±1.8	73±1.9	58±1.6	69±1.8	51±1.6
30	102±2.3	74±2.5	110±2.3	69±2.5	73±1.8	71±2.3	66±1.9

Each value represents the mean(± SD) performance of 3–6 individuals.

Table 3. Absorption efficiency(%) of *Channa striatus* as a function of hormones and dosages (mg/kg live fish).

Dosage	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	90	91	92	92	92	92	92
5	92	92	91	92	91	91	92
10	90	90	92	91	91	92	89
20	89	92	92	91	91	92	89
30	91	90	90	90	91	92	90

Each value represents the mean (±SD) performance of 3–6 individuals.

Hormones: $F(1) 6,24 = 1.08$; $P > 0.25$. Doses: $F(1) 4,24 = 1.43$; $P > 0.25$

$P > 0.05$ is not statistically significant.

all the dosages administered, 20 mg TP or DES dipro and 10 mg DES or DES dipro significantly decreased the feeding rate compared to those receiving sham injection in the respective groups (table 1).

3.2 Absorption

Absorption rate of the individuals receiving different dosages of the tested hormones was found to be dependant on feeding rate. Individuals displaying high feeding rate absorbed the food at a faster rate (table 2). For instance, absorption rate of the individuals receiving Dn was comparatively higher than those receiving other hormones and ranged between 100 and 110 cal/g/day for the different dosages. Correspondingly, individuals whose feeding rate was depressed by DES (5 and 10 mg/kg) and DES dipro (5, 10 and 20 mg/kg) displayed low absorption rate ranging from 58 to 67 cal/g/day. Analysis of the data on absorption efficiency from (table 3) revealed that neither the tested hormones nor the different dosages have any significant influence on absorption efficiency, which ranged from 89 to 92%, and the difference was not statistically significant. This indicates that the steroids do not interfere with the mechanism of food absorption. Understandably, absorption efficiency of the individuals receiving different

Table 4. Weight gain (g) by *Channa striatus* as a function of the different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	3.0±0.2	2.8±0.1	4.4±0.4	2.9±0.2	2.6±0.2	2.7±0.3	2.4±0.3
5	4.3±0.3	3.6±0.3	4.6±0.4	4.3±0.3	2.4±0.2	5.2±0.4	4.3±0.3
10	8.1±0.9	5.2±0.4	5.9±0.6	5.9±0.5	2.8±0.3	4.5±0.5	6.5±0.5
20	3.5±0.2	6.5±0.5	7.9±0.5	5.8±0.5	4.4±0.4	6.0±0.5	2.8±0.2
30	5.3±0.2	5.8±0.3	9.3±0.8	4.3±0.3	6.4±0.5	1.3±0.2	2.6±0.3

Each value represents the mean (±SD) performance of 3–6 individuals.

Table 5. Conversion rate (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	16±1.4	16±1.4	22±0.3	15±0.7	14±0.5	16±1.4	14±1.6
5	24±1.4	19±1.7	27±0.9	24±1.3	13±0.4	29±1.3	22±1.9
10	42±1.5	27±1.1	31±0.6	31±2.0	15±0.9	24±0.4	35±1.3
20	35±1.5	34±1.4	52±0.3	32±1.1	23±0.8	30±1.7	15±0.8
30	28±1.4	37±1.4	61±1.0	23±1.2	35±0.4	7±1.0	15±1.8

Each value represents the mean (±SD) performance of 3–6 individuals.

dosages of the tested hormones does not vary significantly from each other. The data revealed that neither the tested hormones nor dosages influence absorption efficiency (table 3).

3.3 Weight gain and conversion

The initial live weight of the fish used in the feeding experiments in the different hormone series ranged from 9.2 to 10.6 g and the final weight from 11.7 to 19.2 g. Only in the Dn series, weight gain by the fish increased with increasing dosage and of all the tested dosages and hormones 30 mg Dn/kg induced the maximum weight gain of 9.3 g (table 4). In the DES or Ed series, weight gain by the fish increased with dosage up to 10 or 20 mg/kg and declined beyond this level, whereas DES dipro induced maximum weight gain at higher dosages, TP did so only at lower dosages, the lowest weight gain of 1.3 g was recorded for the fish receiving 30 mg T/kg.

Of the tested hormones MT, Ed and Dn induced increased feeding, increased conversion. Individuals receiving 30 mg/kg dosage of MT, Ed or Dn converted the food at the rate of 28, 37 or 61 cal/g/day compared to 16, 16 or 22 cal/g/day in the respective control groups. Individuals in the 20 mg dosage group of T displayed a two-fold increase in the conversion rate over that in the control group (table 5). Although lower dosages of DES and DES dipro suppressed feeding, individuals that received these hormones clearly indicated a tendency to increase

Table 6. Conversion efficiency (%) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	23±2.0	23±2.0	24±2.3	19±2.0	19±2.0	22±0.6	18±0.8
5	30±2.0	23±1.4	24±0.7	33±2.7	20±0.5	30±2.1	33±0.5
10	38±2.0	40±2.5	28±1.7	47±0.6	22±0.5	30±2.2	39±2.2
20	39±2.9	45±1.3	44±2.5	38±2.4	35±1.4	39±1.0	29±2.7
30	25±1.6	46±2.8	49±2.7	30±1.8	43±1.1	8±1.3	21±1.9

Each value represents the mean (±SD) performance of 3–6 individuals.

Table 7. Metabolic units (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	49±2.4	50±2.4	62±1.9	57±1.6	55±2.9	49±1.8	59±2.1
5	49±2.2	57±2.3	73±2.8	43±2.8	46±0.9	58±2.4	40±2.1
10	57±2.6	35±2.9	72±2.1	27±2.8	44±2.7	51±2.2	47±2.9
20	48±2.0	37±1.5	56±2.9	41±1.3	35±2.7	39±2.8	36±2.6
30	74±2.9	37±2.5	49±2.3	46±2.3	38±1.4	64±2.0	51±2.4

Each value represents the mean (±SD) performance of 3–6 individuals.

conversion rate with increasing dosage. The conversion rate of 23 or 35 cal/g/day observed in the 30 mg dosage group of DES or DES dipro was significantly higher than 15 and 14 cal/g/day observed in the respective control groups. Briefly, while a few steroids enhance growth by increasing food consumption, a few other (DES or DES dipro) do so without increasing consumption.

3.4 Conversion efficiency

Data on conversion efficiency of *C. striatus* as functions of the tested hormones and dosages presented in table 6 reveal the following: (a) Administration of 10 or 20 mg dosage of MT, 20 or 30 mg dosage of ED, 30 mg dosage of Dn or DES dipro, 10 mg dosage of DES or TP and 20 mg dosage of T enhances the efficiency to a high level over their respective control groups. (b) Injection of 30 mg/kg of Ed or Dn enhanced the conversion efficiency to 46 or 49%; under similar conditions even 10 mg/kg of DES equally evoked conversion efficiency (47%). (c) The lowest efficiency of 8% is displayed by the individuals receiving 30 mg T dosage showing that at above optimal dosage (>20 mg), T functions as anti-anabolic agent. (d) Estrogens induce better conversion efficiency than androgens; the highest mean efficiency (47%), evoked by estrogens (Ed, Dn and DES) was significantly higher than that (39%) evoked by androgens (MT, T and TP). The lowest efficiency value of 8% observed in the T group was significantly lower than the lowest efficiency (20%) displayed by DES group.

3.5 Metabolic rate

As stated earlier certain dosages of estrogens (30 mg/kg Ed or Dn and 10 mg DES/kg) improved the growth efficiency of *C. striatus*. They achieve this by restricting the energy loss on metabolism and diverting more amount of absorbed food energy for growth compared to those receiving similar dosages of androgens. For instance, *C. striatus* receiving 30 mg/kg dosage of Ed or Dn lost 50 or 45% of the absorbed food energy on metabolism, as against 90 or 77% by those receiving 30 mg/kg dosage of T or TP. At the highest dosage (30 mg/kg), individuals receiving estrogens (ED, Dn, DES and DES dipro) displayed lower metabolic rates (37, 49, 46 and 38 cal/g/day, respectively; table 7) than those receiving androgens (MT, T and TP; 74, 64 and 57 cal/g/day, respectively). A dosage of 5 or 10 mg Dn (estrogen) per kg fish also significantly increased the metabolic rate from 62 cal/g/day in the control individuals to around 72 cal/g/day.

4. Discussion

A scanning of literature on the effect of hormones in fish revealed that earlier studies confined to growth increment (Pickford 1954a, b; Higgs *et al* 1975, 1976, 1977; Bulkley and Swihart 1973); some have also noted the impact of hormones on food consumption (Cowey *et al* 1973; Yamazaki 1976; Markert *et al* 1977; Fagerlund *et al* 1978, 1979; Yu *et al* 1979). The present study provides an integrated approach to explain growth response of fish to hormones. Steroid hormones have been proved to significantly influence feeding in fish. For instance, MT induces appetite and enhances food consumption in *Carassius auratus* (Yamazaki 1976), and *Oncorhynchus kisutch* (Fagerlund *et al* 1978, 1979) while DES depresses appetite and feeding rate in *Salmo gairdneri* (Ghittino 1970; Bulkley 1972; Fagerlund and McBride 1975 a, b). Growth can be increased either by enhancing feeding and/or restricting energy expenditure on metabolism (e.g. 20 mg dosage of Ed; tables 1, 4 and 7). Androgens like MT and T improve growth to a level less than that effected by estrogens through increased feeding. It is obvious that low dosages of estrogens especially DES and DES dipro (5 and 10 mg/kg) are capable of decreasing feeding and increasing growth. Observations of Fagerlund *et al* (1978) on *O. kisutch* reveal that MT administered individuals achieve maximum growth by mobilizing more fat and sparing sacrifice of proteins for energy production. Combined with this, increased feeding by these individuals was also responsible for the significant increase in growth. Hence, it is likely that DES and DES dipro administered *C. striatus* in the present study maximize growth by reserving more protein without consuming significantly more quantity of food. The significantly low rate of metabolism in these individuals supports the above conclusion.

The other estrogens ED and Dn cannot be neglected under the pretext that they induce maximum growth only by increasing the feeding and hence the enhanced cost. Individuals receiving optimal dosage (30 mg/kg fish) of Ed and Dn grew to larger size (15.6 ± 2.4 g and 19.2 ± 2.3 g) than those receiving other estrogens at even time. In aquaculture practices, producing fish of marketable size is of utmost importance (Bulkley 1972; Donaldson *et al* 1979). Therefore, in view of final body weight and marketable size of the individuals Ed and Dn are also useful as growth promoters, despite the high feed cost due to the administration of these hormones. Although the androgens MT and T increase feeding as well as growth of *C. striatus*, growth rate and final body weight (13.5 ± 2.0 and 11.7 ± 1.8 g) of the individuals treated with these hormones are comparatively less than those

receiving Dn or Ed. Hence, based on conversion efficiency and marketable final body size of the test individuals; effectiveness of the tested hormones can be ranked in the following order Dn > Ed > DES > DES dipro > MT > T > TP.

Administration of combination of growth-promoting hormones with contrasting influence on feeding rate may yield surprising results. Combination of growth-promoting appetite stimulators such as Dn or Ed with appetite depressing growth promoters like DES or DES dipro may certainly decrease the feed cost considerably without sacrificing growth. In fact Higgs *et al* (1977) found that a combination of bGH and MT proved to be more effective in promoting growth than either bGH or MT independently. Similar studies involving combination of steroids are required.

Considering the impact of the tested dosages of each hormone on feeding and conversion rates, the hormones can be grouped under 3 different categories. (a) hormones which are effective at high dosages (e.g. Dn Ed: 30 mg dosage) (b) those which are effective at low dosages (e.g. DES, MT, TP: 10 mg dosage) and (c) those which are effective at a low dosage and unfavourable at higher dosage (e.g. T; 5 or 10 mg dosage favourable: 30 mg dosage unfavourable; see tables 1 and 5). Working on *Carassius auratus* Yamazaki (1976) found that 1 ppm dosage of MT was optimal for maximum growth; at 10 ppm dosage the growth rate was significantly less and at 30 ppm dosage the test individuals started losing weight. In the present study, 20 mg/kg dosage of T evoked maximum growth efficiency (39%) while at 30 mg/kg dosage, the efficiency was remarkably reduced to 8%. Reviewing the available information on hormonal enhancement of growth in fish, Donaldson *et al* (1979) proposed that at higher dosages a few steroids exert deleterious effects on various organs and cumulatively cancel the growth-promoting effect. Therefore, administration of arbitrary dosages of hormones may not lead to the realization of the property of the hormone. Optimal dosage for hormones should be fixed separately for each species treating different individuals with a wide range of dosages. For the order of preference of the tested hormones reported above the following are the optimal dosages: Dn: 30 mg; Ed: 30mg; DES: 10mg; DES dipro: 30mg; MT: 10mg pr 20mg; T: 20mg; TP: 10mg. These preferable dosages have been fixed considering the conversion efficiency of the test individuals in the respective groups (table 7).

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Revision of Cassiduloid genus *Gongrochanus* Kier (Echinodermata : Echinoidea) from South Indian Cretaceous deposits

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Abstract. Revision of the genus *Gongrochanus* Kier from Upper Cretaceous beds of South India has revealed the presence of seven species including its type species *G. herschelianus* (M'Clelland). On the basis of phyllode characters the species have been divided into two informal groups. A biometrical analysis by applying the Bartlett's and *F*-test proves the validity of the species described. Coefficients of correlation and regression computed for parameters of length, width and height for *G. herschelianus* are dependent upon one another; that change of length versus width is more regular than that of length versus height. Occurrence of this genus in *Hauriceras remba* zone and *Pachydiscus otacodensis* zone of Kallankurichchi and Ottakkovil formations respectively of Ariyalur Group helps in rectifying its geological age which now can be taken as extending from Lower Maestrichtian to Middle Maestrichtian instead of Senonian.

Keywords. *Gongrochanus*; Cassiduloid; geological range; Maestrichtian; Cretaceous deposits; Echinoidea

1. Introduction

Specimens of *Gongrochanus* collected by M'Clelland (1840) from friable grey sandstones and associated yellow clays and limestones of the Cretaceous age around Cherrapunji (25° - 15', 91° - 44') were placed by him under a new genus *Cyrtoma* describing seven species. Forbes (1846) placed similar specimens from South Indian Cretaceous deposits under *Nucleolites* Lamarck which were subsequently transferred by Stoliczka (1873) to *Stigmatopygus* d'Orb. Since then, while referring to those specimens from Cherrapunji and South India, the name *Stigmatopygus* has been used by many workers like Kossmat (1897), Matley (1929), Spengler (1923), Sastry and Mamgain (1971), Dutta (1974), Bhattacharya and Bhattacharya (1978) and Das *et al* (1981). Only Das Gupta (1920) opined that these specimens differ from *Stigmatopygus* and should be retained under *Cyrtoma* of M'Clelland.

Kier (1962, 1966) in his revision of Cassiduloids proposed a new generic name *Gongrochanus* with *G. herschelianus* (M'Clelland) as its type species; as *Cyrtoma* was pre-occupied in order Diptera (Insecta). Kier (1962, 1966) erroneously designated lectotype for *Gongrochanus herschelianus* (M'Clelland) from Forbes' material (now in British Museum) from South India, whereas a lectotype can only be selected from the original type series of M'Clelland's material. Since efforts to trace the latter material proved in vain, a neotype needs to be established for this otherwise valid species, our material agrees fairly with the redescription of Forbes' material by Kier (1962, 1966).

Therefore, the present authors are getting in touch with Dr Kier, and together designated the neotype and neoparatypes in due course, presuming M'Clelland's material is lost.

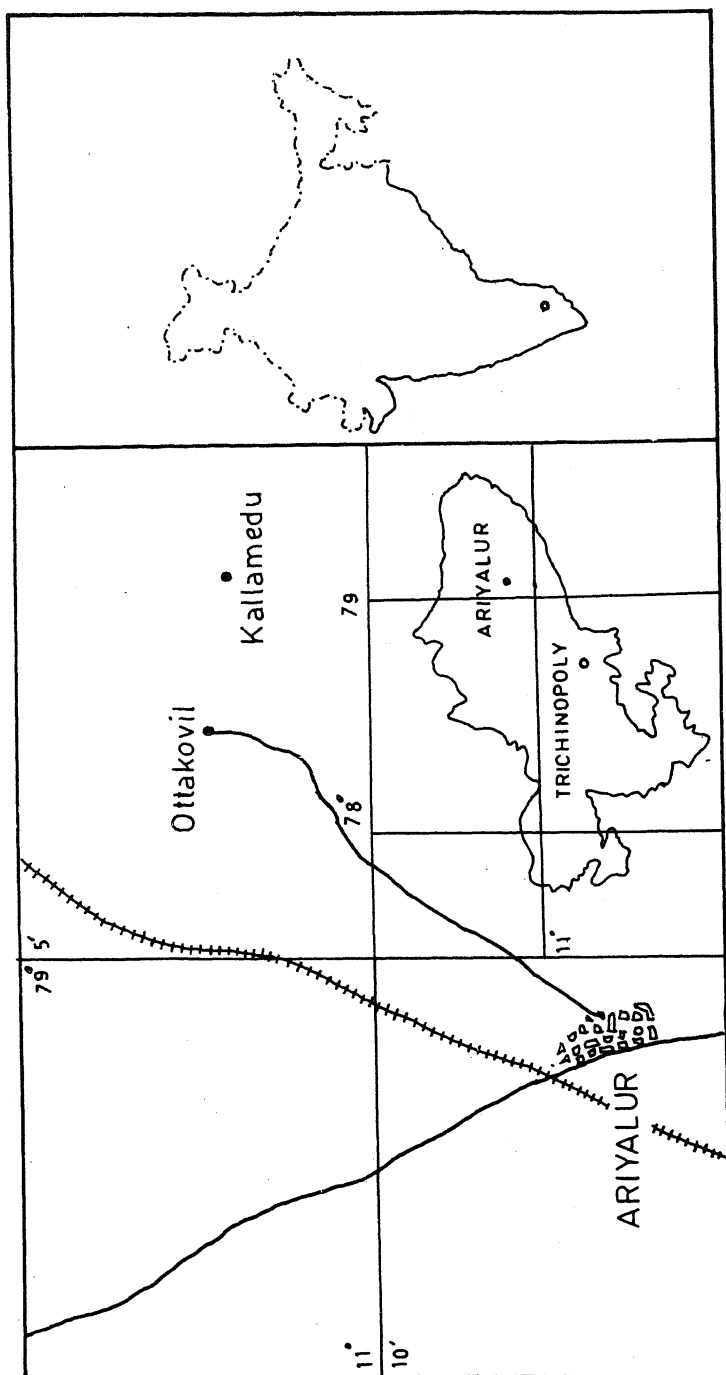


Figure 1. Locality Map.

Out of seven species described by M' Clelland (1840) only *G. herschelianus* has been recognised by Kier (1962, 1966) and Kier and Lawson (1978); and the other names have not been referred to for more than 140 years. Also figures and descriptions given by M' Clelland are far from being adequate for any comparison. Hence, we propose these names be treated as *nomen dubia* and need be rejected as *nomen oblita* (forgotten names) under article 78 (f) of ICZN (1964).

A rich collection of the members of this genus made by us from the Ottakovil area ($11^{\circ} 11'$; $79^{\circ} 07'$) (figure 1) of the South Indian Cretaceous deposits, shows considerable morphological variations warranting a revision. While erecting species more stress is given on variations found in petaloid and phyllode areas which may have direct bearing on the internal anatomy of the soft parts.

The phyllode pattern is characteristically pygurid with two distinctive arrangements of pores from the middle zone. On this basis, two informal groups *viz.* *Herschelianus* and *Ottakovilensis* groups have been proposed. In the former group pores from middle zone are scattered, while in the latter group they are aligned to form a single row (figure 2).

2. Systematic description

Phylum	: Echinodermata
Class	: Echinoidea
Order	: Cassiduloida Claus, 1880
Family	: Fijasidae Lambert, 1905
Genus	: <i>Gongrochanus</i> Kier, 1962

2.1 *Herschelianus* Group: Scattered pores in middle zone.

2.1.a. *Gongrochanus herschelianus* (M' Clelland) (figure 3a-c)

1840 *Cyrtoma herscheliana* M' Clelland, p. 185-187, pl. 5, figure 1-3.

1846 *Nucleolites elatus* Forbes: Forbes, p. 162, pl. 19, figure 1.

1873 *Stigmatopygus elatus* (Forbes): Stoliczka, p. 28, pl. 5, figure 5a-d, non 1-4 and 6, 7, 8.

1920 *Cyrtoma herscheliana* M' Clelland: Das Gupta p. 296.

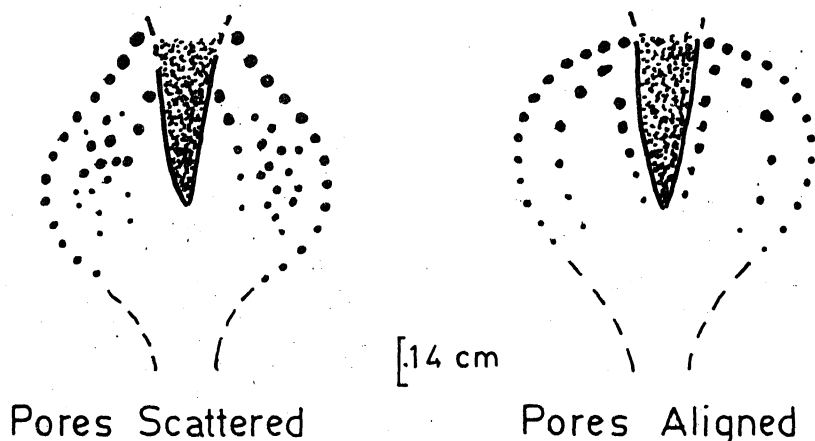


Figure 2. Phyllode patterns.

1923 *Stigmatopygus elatus* (Forbes): Spengler p. 5-7, pl. 1, figures 3a, b.

1962 *Gongrochanus herscheliana* (M'Clelland): Kier, p. 131, pl. 19, figures 4-6, pl. 20, figure 1, text figures 118.

1966 *Gongrochanus herschelianus* (M'Clelland): Kier, P.U. 512, figure 399. 4a-c.

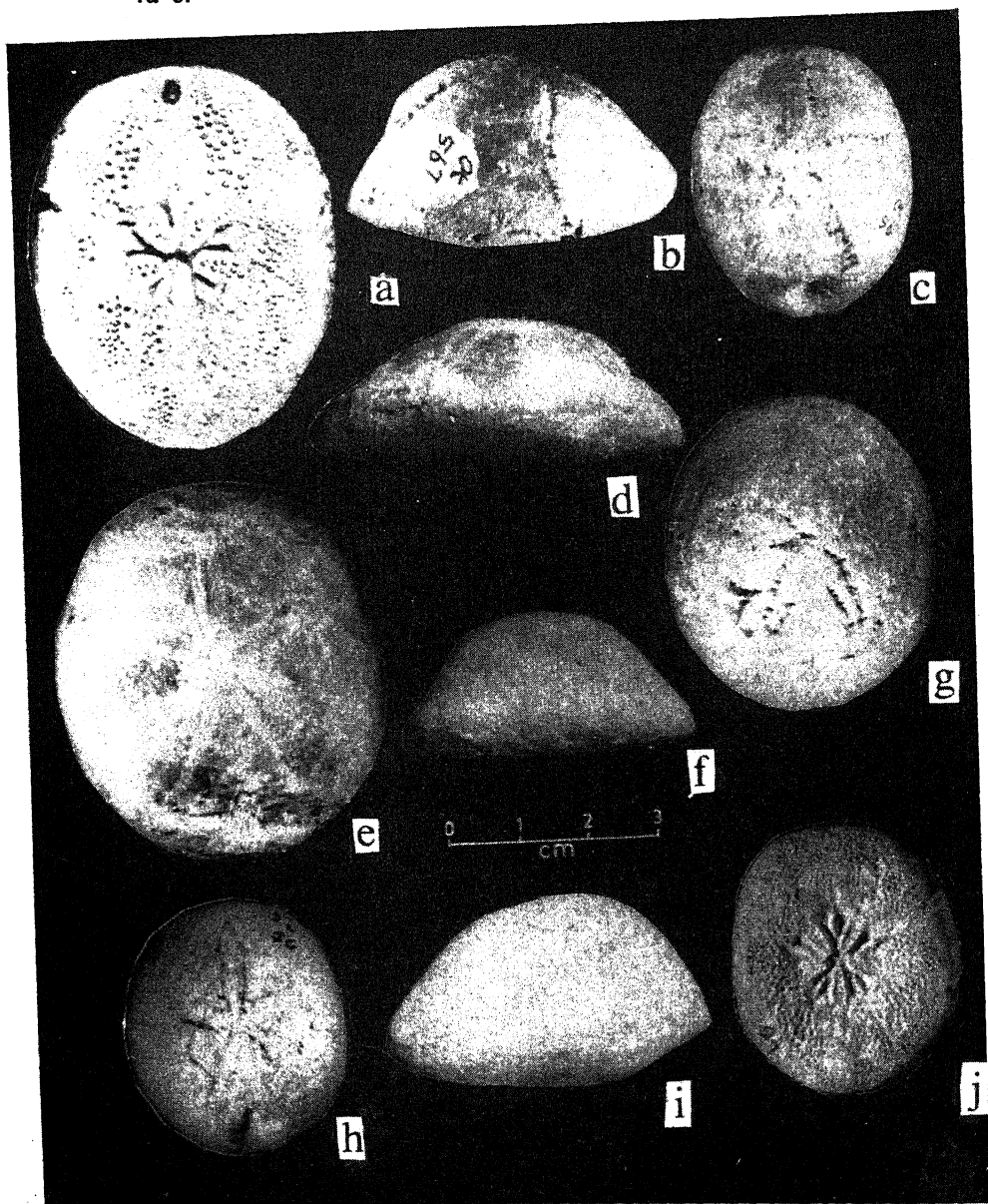


Figure 3. Species of *Herschelianus* group a-c. *G. herscheliana* (M'Clelland) figured specimen No. MACSG 1393 a. oral view b. lateral view c. aboral view d-e. *G. chipionkari* sp. nov. Holotype No. MACSG 1439 d. lateral view e. aboral view; f, g and j *G. stoliczkai* sp. nov. Holotype No. MACSG 1448 f. lateral view g. aboral view j. oral view h, i. *G. kieri* sp. nov. Holotype No. MACSG 1457 h. aboral view i. lateral view.

1978 *Stigmatopygus elatus* (Forbes): Bhattacharya and Bhattacharya, pl. 4; figures 1-3.

Material: 50 Specimens figured specimen no. MACSG 1393 other numbers 1392, 1394 to 1438.

Table 1. Range of number of pores on Ambulacra.

Species	I	II	III	IV	V
<i>Herschelianus</i> Group					
<i>G. herschelianus</i>	23-24	23-24	30-33	20-23	24-25
<i>G. chiplonkari</i>	32-36	30-32	48-50	32-35	30-32
<i>G. stoliczkai</i>	33-35	30-32	38-40	30-32	32-35
<i>G. kieri</i>	28-30	28-30	56-60	30-32	32-36
<i>Ottakovilensis</i> Group					
<i>G. Ottakovilensis</i>	23-24	27-38	35-37	28-30	23-24
<i>G. circularis</i>	26-28	28-30	45-50	30-32	28-30
<i>G. tamilnaduensis</i>	30-32	25-28	40-42	25-28	30-32

Table 2. Range of number of pores on phyllode areas.

Species	Outer row	Middle row	Inner row
<i>Herschelianus</i> Group			
<i>G. herschelianus</i>	10-13	04-07	04-05
<i>G. chiplonkari</i>	09-12	15-17	08-10
<i>G. stoliczkai</i>	18-20	08-10	10-12
<i>G. kieri</i>	17-20	06-08	10-12
<i>Ottakovilensis</i> Group			
<i>G. Ottakovilensis</i>	09-13	05-06	04-06
<i>G. circularis</i>	10-17	08-10	08-12
<i>G. tamilnaduensis</i>	18-20	06-08	10-12

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1392	5.1	4.5	2.8	1.13	1.82
MACSG 1393	5.3	4.5	2.6	1.17	2.03
MACSG 1394	5.0	4.0	2.5	1.25	2.00
MACSG 1395	4.8	3.8	1.8	1.26	2.66
MACSG 1438	4.4	3.5	2.2	1.25	2.00

Description: Test low with distinctly pentagonal ambitus, anteriorly round and posteriorly drawn out. Maximum width at $3/4$ th the length from anterior, posterior slope steeper than anterior, summit at $3/5$ th the length from anterior.

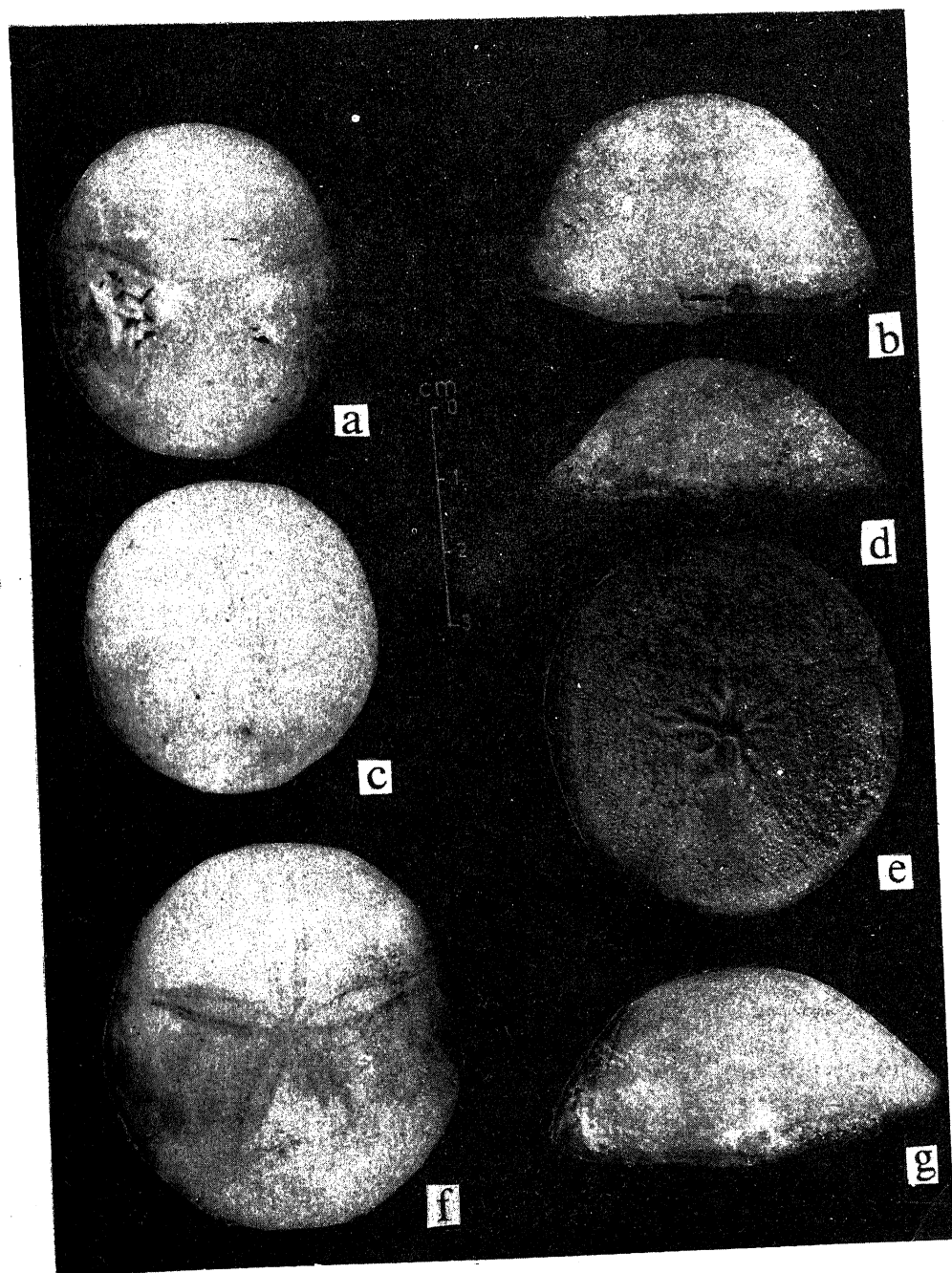


Figure 4. Species of *Ottakovilensis* group. **a, b.** *G. Ottakovilensis* sp. nov. Holotype No. MACSG 1467 **a.** oral view **b.** lateral view **c-e.** *G. circularis* sp. nov. **c.** Holotype No. MACSG 1477, aboral view **d.** Paratype No. MACSG 1478, lateral view **e.** Paratype No. MACSG 1478, oral view **f, g.** *G. tamilnaduensis* sp. nov. Holotype No. MACSG 1487 **f.** aboral view **g.** lateral view.

Ambulacral areas petaloid with 30-33 pores in III ambulacrum (refer table 1 for number of pores on other ambulacra), phyllode pores in three rows, outer slit-like and sinuous along phyllode termination, middle row with scattered round pores and inner row rather straight with only four to five round pores (see table 2 for details of phyllode pores in each species).

Remarks: This species is represented in our collection by a large number of specimens which agree well with figures of *G. herschelianus* given by Kier (1962, 1966). Neotype and neoparatypes will be designated in consultation with Dr Kier since he inadvertently designated lectotype from material other than M'Clelland.

2.1 b *Gongrochanus chiplonkari* n. sp. (figure 3 d, e)

Material: 9 specimens; *Holotype* no. MACSG 1439; *Paratype* nos. MACSG 1440 to 1447.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1439	5.7	4.9	2.1	1.16	2.71
MACSG 1440	5.8	5.5	2.95	1.05	1.966
MACSG 1447	5.5	4.5	2.3	1.22	2.39

Description: Test flattened, low with pentagonal ambitus, adoral surface highly concave. Anterior and posterior slopes nearly equal; summit at half the length from posterior, maximum width in the middle.

III ambulacrum with 48-50 pores; phyllode pores in three rows, outer slit like, sinuous, and 9-12 in number, middle row with scattered pores 15-17 and inner row with 8-10 pores in straight line.

Remarks: This species has low test with highly depressed adoral surface as in *G. stoliczkai*, but the latter has adoral concavity less pronounced, with elliptical outline and summit anterior to the centre, anterior slope steeper than posterior, also petaloid and phyllode pores more in number.

Etymology: The species is named after Prof. G W Chiplonkar for his significant contributions to the Cretaceous Palaeontology of India.

2.1 c *Gongrochanus stoliczkai* n. sp. (figure 3 f, g-j)

Material: 9 specimens; *Holotype* no. MACSG 1448; *Paratype* nos. 1449 to 1456.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1448	5.0	4.2	2.2	1.19	2.27
MACSG 1449	4.1	4.0	2.3	1.02	1.78
MACSG 1450	4.6	3.9	2.3	1.17	2.00
MACSG 1456	5.0	4.6	2.2	1.08	2.27

Description: Test low with elliptical outline, anterior broadly round, posterior slightly protruding, anterior slope steep, posterior gentle, the maximum

width at $1/3$ rd the distance from posterior, summit slightly anterior to centre, adoral surface concave. III ambulacrum with 38-40 pores, 18-20 pores in outer row of phyllode area, middle scattered pores 08-10 in number while the inner row with 10-12 pores.

Remarks: In outline this species appears very close to *G. kieri*, but the latter has flat adoral surface; and much larger number of pores i.e. 50-60 in III ambulacrum.

Etymology: Named after Dr F Stoliczka who did pioneering work in Indian Palaeontology.

2.1d. *Gongrochanus kieri* n. sp. (figure 3h, i)

Material: 10 specimens; *Holotype* no. MACSG 1457; *Paratype* nos. MACSG 1458 to 1466.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1457	5.1	4.2	2.1	1.21	2.4
MACSG 1458	4.9	4.0	2.0	1.22	2.4
MACSG 1466	4.4	3.7	2.1	1.18	2.09

Description: Test low, outline elliptical tapering anteriorly and broad at the posterior, maximum width at $1/3$ rd the length from the posterior, summit in the middle, adoral surface flat, pores in III ambulacrum 56-60. Phyllode area outer zone with 18-20 pores, middle scattered pores 08-10 in number, while the inner row with 10-12 pores.

Remarks: This species resembles close to *G. stoliczkai*, but can be differentiated on the points stated under *G. stoliczkai* (*vide supra*). Another species which can be easily confused with the present species is *G. tamilnaduensis* (*vide infra*) but the pattern of phyllode area in *G. tamilnaduensis* is, however, different, i.e. having aligned pores in the middle zone (see details under *Ottakovilensis*) group (*vide infra*).

Etymology: It is named after Dr P M Kier of Smithsonian Institute, U.S.A.

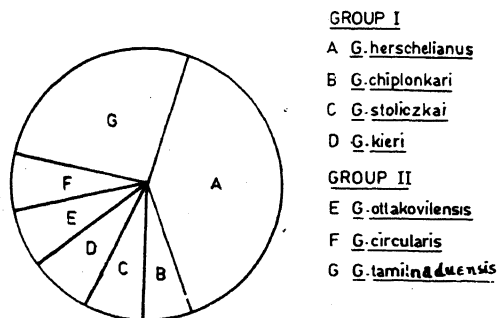


Figure 5. π -diagram showing population of erected species.

2.2 *Ottakovilensis* Group :

Aligned pores in the middle zone. This informal group comprises three species viz., *Gongrochanus ottakovilensis*, *G. tamilnaduensis* and *G. circularis*.

2.2a. *Gongrochanus ottakovilensis* n. sp. (figure 4a, b)

Material: 12 specimens: *holotype* no. MACSG 1467. *Paratype* nos. 1468 to 1478.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1467	5.5	4.1	3.1	1.34	1.77
MACSG 1458	3.6	3.1	2.5	1.16	1.44
MACSG 1478	6.0	4.6	3.2	1.30	1.87

Description: Test tall, elliptical with sides parallel, anteriorly obtusely round, posteriorly slightly drawn. Maximum width in the middle and summit in the centre, posterior slope steeper than anterior. Number of pores on ambulacrum III 35-37 and phyllode pores in three distinct rows, outer ones with 09-13 pores, middle parallel to outer ones and 05-06 in number, the inner ones 04-06 in number forming a straight line.

Remarks: This species can be separated from its two allies i.e. *G. circularis* and *G. tamilnaduensis* by its tall test, and less number of ambulacral pores in ambulacrum III, and in outer zone of phyllode area.

Etymology: It is named after the locality.

2.2b. *Gongrochanus circularis* n. sp. (figure 4c, d, e)

Material: 10 specimens; *Holotype* no. MACSG 1477. *Paratype* nos. 1478 to 1486.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1477	4.4	3.7	2.1	1.18	2.09
MACSG 1478	5.0	4.6	2.3	1.08	2.17
MACSG 1479	5.0	4.5	2.1	1.11	2.38
MACSG 1486	3.9	3.7	2.0	1.05	1.95

Description: Test low, ambitus circular, posterior margin sometimes insignificantly drawn out; adoral surface flat, ambulacral areas distinctly raised above the general surface; summit centrally situated. Number of pores in III ambulacrum 45-50, phyllode pores typical of group II being in three zones. Outer zone with 10-17 pores, middle with 08-10 pores and inner with 8-12 pores.

Remarks: Since it is circular in outline, with adoral surface flat and centrally placed summit, it has discoidal appearance and ambulacral areas distinctly raised above the general surface.

Etymology: The name is derived from the circular ambitus, a characteristic feature of this species.

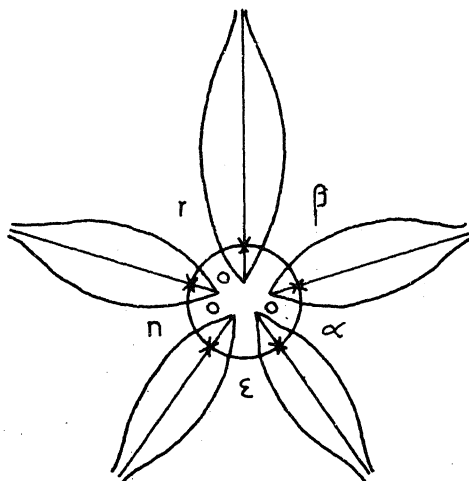


Figure 6. Schematic representation of interpetaloid angles.

2.2c. *Gongrochanus tamilnaduensis* n. sp. (figure 4f, g)

Material: 42 specimens; Holotype no. MACSG 1487. *Paratype* nos. 1488 to 1528.

Dimensions: (in cm)

Sp. No.	L	W	H	L/W	L/H
MACSG 1487	5.8	4.8	2.6	1.20	2.23
MACSG 1488	5.0	4.3	1.7	1.16	2.94
MACSG 1489	5.3	4.4	2.4	1.20	2.20
MACSG 1528	5.2	4.7	2.4	1.10	2.16

Description: Test medium in height, sub-pentagonal to pentagonal in outline, anterior side with a gentle slope than the posterior; maximum width at half the length and nearly central summit. Adoral surface flat. Number of pores in ambulacrum III 40-42, phyllode outer zone sinuous with 18-20 pores, middle zone with 06-08 pores and inner zone with 10-12 pores.

Remarks: This species differs from *G. circularis* in having pentagonal outline, while it has higher L/H ratio as compared to *G. ottakovilensis*.

Etymology: It is named after the state of Tamilnadu in which these fossils occur.

2.3 *Locality and horizon*

All the species described above come from gray to white coarse grained calcareous sandstones of Ottakovil Formation of Ariyalur Group exposed east of Ottakovil.

3. Discussion

3.1 *Comparison with Stigmatopygus*

Gongrochanus can easily be distinguished from *Stigmatopygus* by its supramarginal periproct situated in a longitudinal groove and presence of a prominent

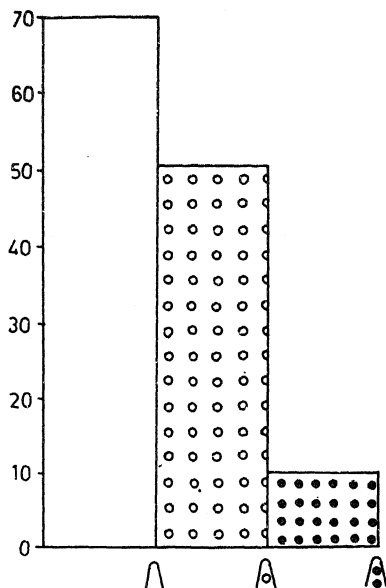


Figure 7. Variation of tuberculation on V tooth.

bulge on the median area of each phyllode. Kier (1962 p. 132) states, "Striking feature in *Gongrochanus hershelianus* is prominent bulge in the median area of each phyllode. This feature is not present on any of the species of *Stigmatopygus* or as far as I know in any other echinoid"

3.2 Petaloid pores (table 1)

Pores in petaloid region vary in number in individual specimens, but can be grouped by fixing certain limits and it has been found that this grouping when taken with other features helps to identify the species.

3.3 Phyllode pores (table 2)

The set of pores developed in the phyllode area appears more significant at generic level (Kier 1962, 1966).

A careful study of population of the species described here reveals that larger number of specimens fall in *Herschelianus* group than in *Ottakovilensis* group (figure 5).

The evolutionary trend of pygurid phyllode may be towards that of *Ottakovilensis* group, where there is reduction in the number of pores, accompanied by their alignment.

A critical study of three hundred and odd specimens belonging to this genus, with a view to find out a basis of classification of the species described here has revealed the pattern of pores in the phyllodes; according to which, these species could be placed in two informal groups, *Herschelianus* and *Ottakovilensis*.

3.4 Ambital geometry and nature of adoral surface

Further speciation can be made on the characters like outline of the ambitus, nature of adoral surface and interambulacral angles, etc.

Most of the specimens described here generally have pentagonal to oval outline at ambitus, but *G. circularis* is characterised by a distinctly circular outline.

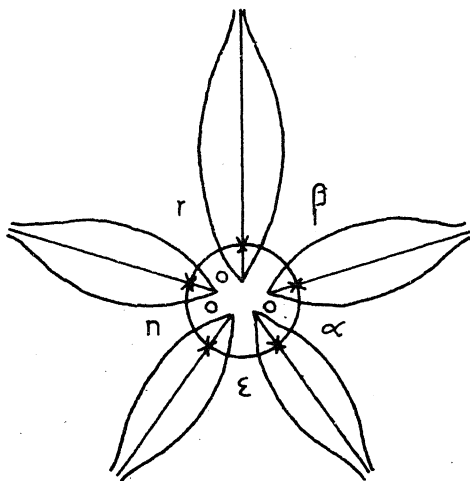


Figure 6. Schematic representation of interpetaloid angles.

2.2 c. *Gongrochanus tamilnaduensis* n. sp. (figure 4f, g)

Material: 42 specimens; Holotype no. MACSG 1487. *Paratype* nos. 1488 to 1528.

Dimensions: (in cm)

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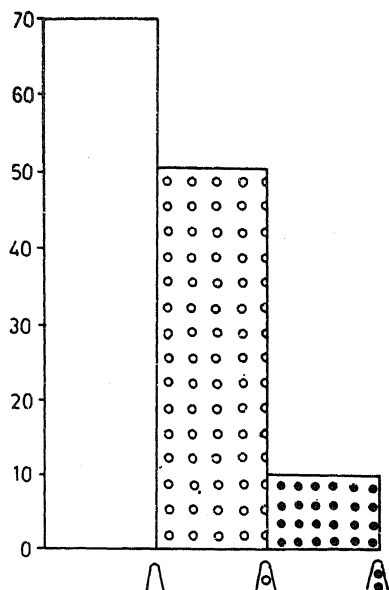


Figure 7. Variation of tuberculation on V tooth.

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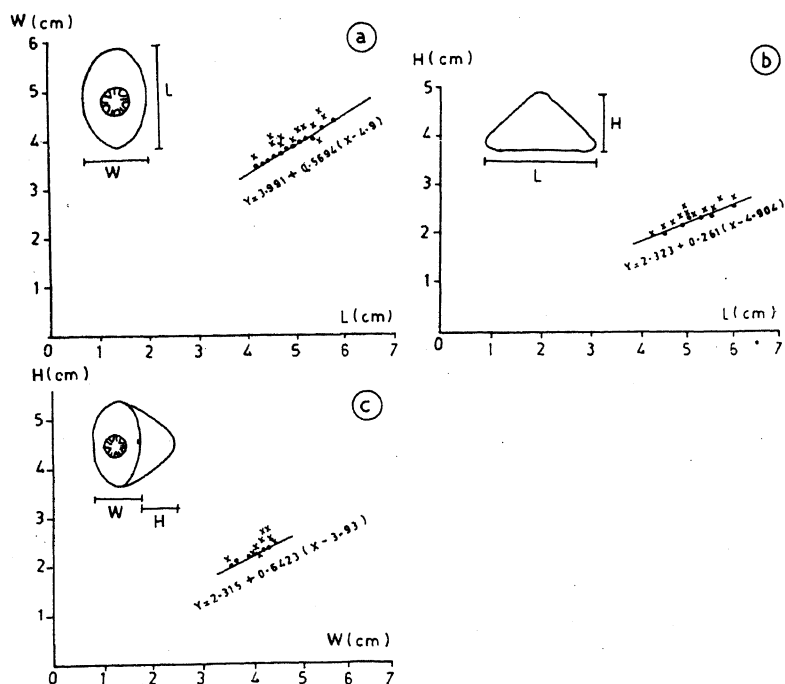


Figure 8. Regression lines of morphometric parameters a. width on lengths b. height on length c. height on width.

G. chiplonkari and *G. stoliczkai* have their oral surface depressed thus differing from other species. This feature cannot be considered as just a variation, but appears to have an evolutionary significance since it must have affected the internal anatomy of the animals, also consequent to the external build.

3.5 Ambulacral angles

As conceived from figure 6 and table 3 species may have variable interpetaloid angles without any pattern which cannot be linked up with *L*, *W* and *H*. Thus *G. chiplonkari* and *G. stoliczkai* though appear almost identical in external morphology including concave oral surface have their posterior inter-petaloid angle (ϵ) highly distinctive and can be isolated by visual examination.

3.6 Tuberculation

One more variation which needs attention is tuberculation on the fifth tooth. On the other four teeth, the number and arrangement of tubercles are fairly constant, but on the fifth tooth three variations have been noticed, irrespective of the species and growth stage (figure 7). In some specimens, the area of the fifth tooth is completely naked; some have only one tubercle at the distal end while some are with two or more tubercles, covering the entire surface of the tooth. Observations made on about 130 specimens reveal that the frequency of the naked tooth is highest. The advantage or disadvantage of the absence or presence of spines on the fifth tooth is not yet sufficiently understood.

4. Age

The specimens of *Gongrochanus* described here come from the Ottakovil Formation of Ariyalur Group. Recently a few specimens of this genus have also

Table 3. Interpetaloid angles (in degrees).

Species	α	β	γ	η	ξ
<i>Herschelianus</i> Group					
<i>G. herschelianus</i>	58-60	89-91	89-90	47-49	69-70
<i>G. chiplonkari</i>	78-80	52-53	91-93	72-75	61-63
<i>G. stoliczkai</i>	68-70	79-83	75-77	89-92	40-42
<i>G. kieri</i>	77-79	80-82	80-81	67-70	52-55
<i>Ottakovilensis</i> Group					
<i>G. Ottakovilensis</i>	90-93	60-63	47-50	89-90	50-53
<i>G. circularis</i>	62-66	78-80	58-60	90-94	58-60
<i>G. tamilnaduensis</i>	80-82	70-74	68-70	76-78	54-56

Table 4. Bartlett's test for the genus *Gongrochanus*.

Morphometric parameter	χ^2 with six degree of freedom	Table values of six degrees of freedom at 0.05 probability
Length	1.2674086	12.59
Width	1.99997665	12.59
Height	2.7721758	12.59

Table 5. F-Test for the genus *Gongrochanus*.

Morphometric parameter	Degrees of freedom	Table value at 0.05 probability	Calculated value	Inference
Length	F ⁶ 141	3.7	4.9933094	Means of morphometric parameters are 99% significantly different
Width	F ⁶ 145	3.7	6.5846112	
Height	F ⁶ 145	3.7	4.03476673	

Table 6. Co-efficient of correlation (r) and regression: (b) computed for different combinations of morphometric parameters of *G. herschelianus*.

Combinations of morphometric parameters	r	b
Length <i>vs</i> width	0.85192977	0.569431399
Length <i>vs</i> height	0.56013788	0.261080092
Width <i>vs</i> height	0.80424293	0.0642350795

been collected from the Kallankurichchi Formation of the same Group from near Kallankurichchi. Sastry *et al* (1968, 1972) have considered these two formations to range from Lower Maestrichtian to Middle Maestrichtian on the basis of *Hauriceras rembda* zone and *Pachydiscus otacodensis* zone.

The original specimens of this genus reported by M'Clelland (1840) were from Assam Cretaceous deposits. Bhattacharya and Bhattacharya (1978) made a fresh

collection from these areas and reported specimens of *Gongrochanus* as *Stigmatopygus elatus* proposing a new zone *Anisoceras* – *Stigmatopygus* assemblage zone within the Mahadek Formation. The Mahadek Formation ranges in age, from Campanian to upper Maestrichtian (Bhattacharya and Bhattacharya *op cit.*). However, they have not given the precise age of *Anisoceras* – *Stigmatopygus* zone which is developed in the upper part of Mahadek Formation. Hence in the light of a firm evidence obtained from biostratigraphic zonations of South Indian Cretaceous deposits the genus *Gongrochanus* ranges from Lower Maestrichtian to Middle Maestrichtian. Thus, the Senonian age proposed by Kier (1962, 1966) and Kier and Lawson (1968) needs a rectification as above. It may be mentioned here that the genus *Stigmatopygus* d'Orbigny *Sensu stricto* is so far known to occur in Europe and Africa (Kier 1962, 1966; Kier and Lawson 1978). As against that the *Gongrochanus* is restricted only to Indian sub-continent being reported from Kallankurichchi and Ottakovil Formations from South Indian Cretaceous deposits in Tamilnadu and the Mahadek Formation from Meghalaya. Since the genus *Stigmatopygus* is not known to occur in India, all the specimens described under it are infact of *Gongrochanus* Kier. Consequently *Anisoceras* – *Stigmatopygus* assemblage zone of Bhattacharya and Bhattacharya (1978) has to be redesignated as *Anisoceras* – *Gongrochanus* assemblage zone and precise age of it will have to be established after a critical study of the fauna of this zone.

5. Key

Following is the key to identify species of *Gongrochanus*.

1. Phyllode area with middle zone

having scattered pores

Informal *Herschelianus* group

G. herschelianus,
G. kieri, *G. stoliczkai*,
G. chipionkari

1. Phyllode area with middle zone

having aligned pores

Informal *Ottakovilensis* group

G. Ottakovilensis,
G. tamilnaduensis,
G. circularis

5.1 Informal *Herschelianus* Group

1. Test tall

G. herschelianus

1. Test dwarf

2. Adroally plane

G. kieri

2. Adroally concave

G. chipionkari
G. stoliczkai

3. Posterior ambulacral angle of divergence narrow (40° – 42°)

G. stoliczkai

3. Posterior ambulacral angle of divergence broad (61° – 63°).

G. chipionkari

5.2 Informal *Ottakovilensis* Group

1. Test circular

G. circularis

1. Test not circular

2. Phyllode pore in outer zone more
2. Phyllode pore in outer zone less

G. tamilnaduensis
G. Ottakovilensis

6. Biometrics

All the seven species described here come from the same locality and horizon representing a heavy fossil population of *Gongrochanus*. A casual inspection of six hundred and odd specimens collected systematically revealed that this population is highly diversified, and it would not be proper to place them under a single species. With a careful examination of various morphological parameters seven species have been recognized as given in the key. Further to strengthen the validity of these species two important biometric tests have been applied. These are Bartlett's and *F*-tests (Woolf 1968; Freneix 1979). By Bartlett's test (table 4) homogeneity of variances of these species is tested. The morphometric parameters *viz.* length, width and height have been chosen for this test.

It has been found that Chi-square is sufficiently less than the table values and hence its null hypothesis of homogeneous variances is accepted. Thus the new species maintain their entity.

The second test applied is the *F*-test (table 5) by using the same parameters of length, width and height. The null hypothesis assumes that the species have similar means of their morphometric parameters. Contrary to this assumption the test shown higher values than the table values, rejecting the null hypothesis. This again proves the validity of being the new species.

The coefficients of correlation and regression are computed for the different combinations of morphometric parameters of *G. herschelianus* (table 6). It indicates that the changes of *L*, *W*, and *H* are dependent upon one another and the change of length versus width is more regular than that of length versus height (figures 8 a, b) and the change of width versus height is more akin to that of length versus width (figure 8c).

Recently, Das *et al* (1981) have made a morphometric study of *Stigmatopygus elatus* (Forbes) (now *G. herschelianus*). In using this taxonomic designation they have evidently overlooked or are unaware of the taxonomically decisive significance of phyllode and petaloid areas. Similarly, specimens described by McClelland (1840), and Bhattacharya and Bhattacharya (1978) as *Cyrtoma* and *Stigmatopygus elatus* (Forbes) respectively belong to *Gongrochanus*. Hence even in the absence of characters of oral region the specimens of Das *et al* can be placed under *Gongrochanus*.

Before adopting any statistical approach due attention should be given to the characters which are diagnostic at generic level. In the case of *Gongrochanus* teeth like bulges on phyllode areas are unique feature peculiar to this genus. The other character which may not be morphometrically important, but genetically diagnostic is the alignment of pores on phyllode area which helped us to split the South Indian specimens of this genus into two informal groups. Thus, it will be worthwhile in first ascertaining all such features to decide correct taxonomic position and before subjecting any material to desired statistical exercises, otherwise it will result in faulty exercises due to wrong evaluation of characters of biological significance.

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Life history of sexuparae and sexual morphs of woolly apple aphid (*Eriosoma lanigerum* Hausmann)

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Abstract. The life history of sexuparae and sexual morphs of woolly apple aphid (*Eriosoma lanigerum* Hausmann) is reported from Simla Hills. Sexuparae are alate parthenogenetic viviparae and produce the sexuals (males and oviparae). The reproductive potential of sexuparae is much lower as compared to that of virginoparae. Sexuals are apterous and have degenerated mouth parts. An ovipara lays only one egg. All these morphs undergo four moults resulting in five instar stages.

Keywords. Woolly aphid; sexuparae; sexuals; morphs; virginoparae; *Eriosoma lanigerum*.

1. Introduction

Woolly apple aphid (*Eriosoma lanigerum* Hausmann) is a native of North America where it undergoes a regular alternation of a bisexual generation with a series of parthenogenetic generations (Baker 1915). Elm is the primary host of this insect pest while apple and related plants are secondary hosts. However, in other apple-growing regions of the world, the behaviour and biology of woolly apple aphid differ considerably because of the absence of its primary host elm (*Ulmus americana*) and also due to different environmental conditions.

In India, this pest has completely adapted to apple plants and causes great damage every year. Although the biology of virginoparae morphs (apterous parthenogenetic females) has been studied by some workers (Rahman and Khan 1941; Lal and Singh 1947; Gautam 1981), very little is known about the biology of sexuparae and sexual morphs of this insect pest. The present authors had reported the occurrence of sexuparae and sexual morphs in Simla Hills (Gautam and Verma 1982) and this paper deals with the life history of these morphs.

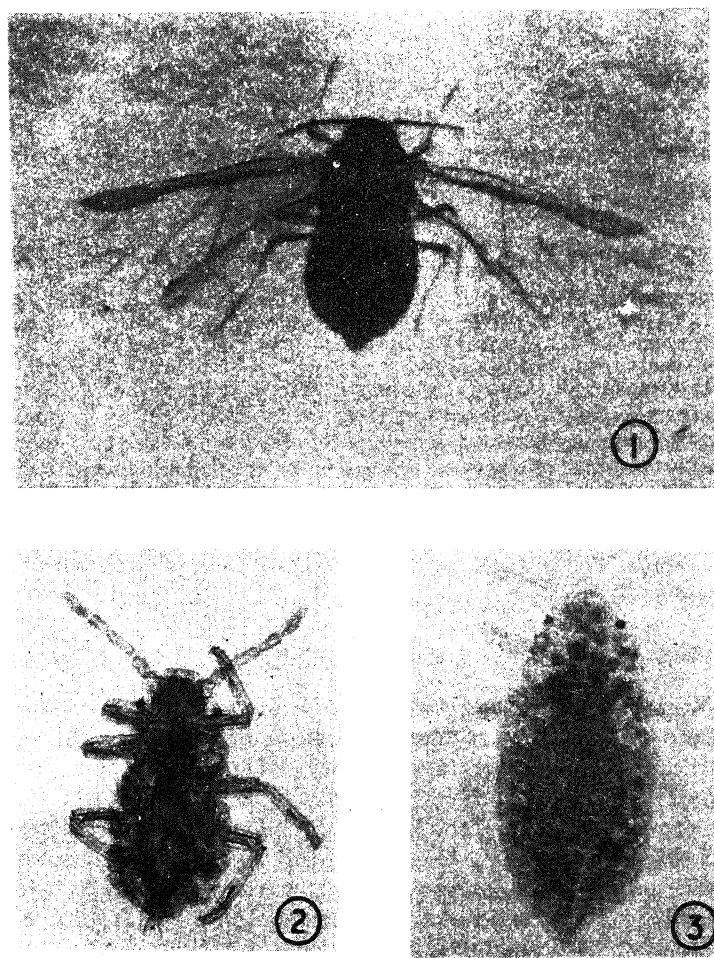
2. Material and methods

The culture of woolly apple aphid was maintained on apple nursery plants in the laboratory and the life history of sexuparae and sexual morphs was studied under 8 L: 16 D photoperiod from August to October. The temperature ranged from 19 to 23.5°C (mean temperature 21.78°C) during this period. The various aspects of life history of these morphs were recorded daily at 0800, 1200 and 1600 hrs. The number of instars of these morphs was determined by examining the exuviae with a magnifying lens. During reproductive period, the progeny produced by a female was also recorded daily.

3. Results and discussion

3.1 Sexuparae

In the fields (apple orchards), sexuparae appear from late July to November with



Figures 1–3. *Eriosoma lanigerum*. 1. Sexupara ($\times 20$) 2. Male ($\times 75$) 3. Ovipara ($\times 75$).

maximum population in September–October. These are alate, parthenogenetic, viviparous and male–female producers. Each individual undergoes four moults and the last moult gives rise to adult alate sexupara. It is difficult to differentiate the first and second instars of the alate sexuparae from that of apterous virginoparae as the wing pads are not clearly visible even with magnifying lens but in the third and fourth nymphal instars; the wing pads are very prominent and these two morphs can be differentiated on the basis of this morphological character.

The adult alate sexuparae have six segmented antennae bearing secondary rhinaria on the last four segments. The compound eyes are multifaceted and dark black. There are two pairs of wings; the forewings possess a prominent pterostigma. The media is forked and not traceable near the base (figure 1). The hind tarsi are two segmented; the proximal segment is triangular with two long hairs and the distal segment bears a pair of claws.

The average duration of first, second, third and fourth nymphal instar is 3.95, 2.7, 2.75 and 4.25 days respectively. The total nymphal period ranges from 12–15 days (average 13.65) (table 1). The adult individuals live for 4 to 7 days (mean value 5.4 days) and the progeny produced ranges from 4 to 8 sexuals (average 5.3

Table 1. Duration of developmental stages, total longevity and fecundity of sexuparae and sexual morphs of woolly apple aphid on apple plants (8L:16D).

Morph*		Nymphal period (in days)			Total nymphal period (in days)	Adult life period** (in days)	Total life period (in days)	Progeny produced by a female
		First instar	Second instar	Third instar	Fourth instar			
Sexupara	Mean	3.95	2.70	2.75	4.25	5.40	19.05	5.30
	SE***	±0.20	±0.15	±0.15	±0.20	±0.34	±0.49	±0.40
Ovipara	Mean	2.45	1.20	1.55	2.20	5.50	12.90	One egg
	SE	±0.14	±0.11	±0.16	±0.24	±0.80	±0.80	-
Male	Mean	1.45	1.65	2.25	1.65	3.30	10.30	-
	SE	±0.14	±0.18	±0.11	±0.13	±0.34	±0.47	-

* Data represented for ten individuals of each morph. ** In case of ovipara, it is the period between the last moult and the egg laying. *** (±) Standard error about the mean.

nymphs). The total life period of sexupara ranges from 17 to 22 days (average of 19.05 days) (table 1). The abdomen of the sexupara gets shrunk after laying the sexuals. The fecundity rate of sexupara recorded in the present investigations is very low as compared to that of virginopara (Gautam 1981) and this may possibly be because of the physiological changes in sexuparae due to morph differentiation.

3.2 Sexuals

Sexuals (males and oviparae) are the progeny of sexuparae. These are apterous and undergo four moults before they become sexually mature. Mouth parts are degenerated (atrophied) and the antennae are five-segmented in all the instars (nymphs as well as adults) of both the morphs. The last two segments of antennae possess primary rhinaria. In the sexuals, eyes are three-faceted and the tarsi of all the legs are one-segmented having three pairs of long capitate hair, a pair of long hair and a pair of claws. Males (figure 2) are smaller in size and the body is dark brown whereas oviparae (figure 3) are reddish brown and in both the sexual morphs, the antennae and legs are transparent with yellowish tinge.

In males, the average duration of first, second, third and fourth nymphal instar is 1.45, 1.65, 2.25 and 1.65 days respectively. The total nymphal period ranges from 6 to 9 days (mean value 7 days). The total life period ranges from 8-12 days with an average value of 10.3 days (table 1). Males are very active as compared to oviparae.

In oviparae, the average duration of first, second, third and fourth nymphal instar is 2.45, 1.2, 1.55 and 2.2 days respectively. The total nymphal period ranges from 7 to 8 days (mean value 7.4 days). The period between the last moult and the egg laying ranges from 3 to 11 days (mean value 5.5 days) and the total life period ranges from 10 to 16 days with an average value of 12.9 days (table 1). Earlier, Fotedar and Kapur (1943) also reported that in Kashmir valley a male survives for about 12 days and an ovipara lives for about 20 days.

Ovipara lays only one egg which in the adult ovipara occupies the whole abdomen and dies in the process of egg laying. The egg is reddish brown when freshly laid but turns dark brown later on.

The sexuals (nymphs as well as adults) have degenerated mouth parts but still they survive and undergo so many physiological changes during their development such as moulting, maturing etc. According to Dunn (1959), the sexuals of *Pemphigus bursarius* L. are also arostrate and survive presumably on the stored reserves of the body. These sexuals undergo moulting before they become sexually mature. Hille Ris Lambers (1966) also discussed that in Eriosomatinae, the oviparae have no functional mouth parts, show a negative growth through a number of moults and after mating with similar dwarfy males, lay only one egg while in Fordini, the oviparae die with one egg inside the body.

In North America, where this insect pest has holocycle on elm (primary host) and apple plants; the sexuparae are produced on apple plants, from where these migrate to elm and produce sexuals there (Baker 1915). However, in India this pest has completely adapted to its secondary host (apple plants) and the sexuparae produce sexuals on these plants.

Fluiter (1948) reported that the sexuparae of woolly apple aphid may also produce, besides males and oviparae, the intermediates with fully developed mouth parts like those of viviparae or half developed mouth parts. However, in the present investigation the progeny produced by a sexupara consisted of males and oviparae only.

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Laboratory evaluation of poison carrier for the control of Indian gerbil, *Tatera indica* Hardwicke

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Abstract. *Tatera indica*, a common rodent pest of Indian agriculture, quickly develops poison aversion and bait-shyness towards zinc phosphide, the only acute rodenticide known to farming community. For managing residual population of field rodents, alternate baits are, therefore, required. The present study gives the results of bait preference studies conducted on *T. indica*.

Keywords. *Tatera indica*; poison carrier; bait preference; additives; choice test; Indian gerbil; energy budget.

1. Introduction

Poisoning is the humane method of rodent control (Fitzwater and Prakash 1977) but implementation of the actual management technology requires thorough knowledge of an appropriate bait-carrier of poison. Since rodents of Indian agriculture develop poison aversion and bait-shyness towards zinc phosphide, the only acute stomach poison widely used for field rodent management in India (Prakash and Jain 1971; Prakash *et al* 1975), it becomes more pertinent to work out new effective poisons as well as alternate poison carriers.

The present investigation embodies results of bait preference studies conducted on *Tatera indica*, a common rodent pest of Indian agriculture.

2. Material and methods

Bait preference test was conducted with 10 gerbils each lodged in individual laboratory cages. In free choice tests (multiple choice), three baits, selected from 10 candidate baits with aid of random number tables, were exposed to every gerbil for six days continuously. Consumption of each bait was measured daily and replaced with a new series of baits the following day. In no choice or single choice tests, common foodgrains like bajra, jowar and wheat, were exposed singly to each gerbil. Average daily intake (ADI) of each bait was calculated at the end of each test, after transforming the absolute consumption values to g/100 g body weight of the individual gerbil. Vegetable oils, salt and sugar were also added and exposed. Water was available *ad libitum*. All tests were conducted according to the following scheme. (a) Free-choice tests—whole grains; cracked grains; whole grains *vs* cracked grains; whole grains *vs* flours; whole grains *vs* roasted grains; whole grains + oils; whole grains + oils + salt and sugar; cracked grains + oils; cracked grains + oil + salt and sugar; flours + oils; flours + oil + salt and sugar (b) No choice tests—bajra; jowar; wheat; and (c). Top ranking baits from each test. Other patterns of evaluation are as described earlier (Jain *et al* 1974, 1975; Anon 1981).

Table 1. Consumption of food grains (g/100 g body weight, Mean $\pm$ S.E.) by *T. indica*.

Foodgrains	Whole grains	Energy (kJ)	Cracked grains	Energy (kJ)
Bajra (<i>Pennisetum typhoides</i>)	3.79 $\pm$ 0.62	57.4	3.26 $\pm$ 0.30	49.3
Barley (<i>Hordeum vulgare</i>)	4.65 $\pm$ 0.53	65.6	3.23 $\pm$ 0.33	45.5
Bengal gram (<i>Cicer arietinum</i>)	3.80 $\pm$ 0.53	57.4	2.27 $\pm$ 0.34	34.3
Guar (<i>Cyamopsis tetragonoloba</i>)	0.41 $\pm$ 0.03	—	1.68 $\pm$ 0.29	—
Jowar (<i>Sorghum vulgare</i>)	1.09 $\pm$ 0.12	15.9	1.34 $\pm$ 0.23	19.6
Maize (<i>Zea mays</i>)	5.72 $\pm$ 0.69	82.1	2.14 $\pm$ 0.31	30.7
Green gram (<i>Vigna radiatus</i>)	3.07 $\pm$ 0.53	43.0	3.33 $\pm$ 0.30	46.7
Moth (<i>Phaseolus aconitifolius</i>)	3.52 $\pm$ 0.66	48.7	3.47 $\pm$ 0.49	48.6
Black gram (<i>Phaseolus radiatus</i>)	2.12 $\pm$ 0.36	29.7	2.25 $\pm$ 0.30	32.7
Wheat (<i>Triticum aestivum</i>)	6.05 $\pm$ 0.80	87.9	3.45 $\pm$ 0.31	50.1
average (kJ)/100 g body weight/24 hr		54.20 $\pm$ 18.06		39.72 $\pm$ 13.24

3. Results

3.1 Whole grains

Wheat (*Triticum aestivum*), maize (*Zea mays*) and barley (*Hordeum vulgare*) were preferred to all other grains tested (table 1). However, there was no significant difference between consumption of wheat and maize; and wheat and barley ($t=0.31$ and 1.45 respectively). Gerbils preferred large-sized maize or long grains of wheat as compared to small and round grains of pearl millet (*Pennisetum typhoides*), jowar (*Sorghum vulgare*), green gram (*Vigna radiatus*) and moth (*Phaseolus aconitifolius*).

3.2 Cracked grains

Among cracked grains, gerbils liked moth > wheat > green gram > pearl millet > barley without any significant difference between their consumptions ($t=0.1, 0.2, 0.4$ and 0.5 respectively) as compared to top-rated moth (table 1).

3.3 Whole grains vs cracked grains

Cracked grains were clearly preferred to whole grains except bajra (table 2). Gerbils choose wheat followed by maize and bengal gram (*Cicer arietinum*) without any significant difference between their consumptions ($t=0.3, 0.5$ respectively). Antelope rats too maintained preference for the same grains except that bengal gram replaced barley (table 2).

3.4 Whole grains vs flours

Among flour forms bengal gram > wheat > maize was favoured without any statistically significant difference between their respective consumptions ($t=0.31, 0.47$, table 2). Interestingly, these top ranking foodgrains change their positions in the preference hierarchy with change in their forms or textures.

3.5 Whole grains vs roasted grains

Rodents clearly preferred roasted grains to dry whole grains in as much as that the relative consumption of roasted jowar (*Sorghum vulgare*) was significantly more from its dry grain form ($t=6.46, P>0.001$, table 3).

Table 2. Relative consumption (g) of various forms of food grains by *T. indica*.

Food items	Whole grains vs cracked grains			Whole grains vs flours		
	Whole Mean $\pm$ SE	Energy (kj)	Cracked Mean $\pm$ SE	Whole Mean $\pm$ SE	Energy (kj)	Flours Mean $\pm$ SE
Bajra	2.16 $\pm$ 0.46	32.7	2.12 $\pm$ 0.50	2.01 $\pm$ 0.49	30.4	1.24 $\pm$ 0.28
Barley	1.88 $\pm$ 0.39	26.5	2.08 $\pm$ 0.31	2.92 $\pm$ 0.36	41.2	1.90 $\pm$ 0.42
Bengal gram	2.47 $\pm$ 0.36	41.5	3.26 $\pm$ 0.55	3.56 $\pm$ 0.54	53.8	3.86 $\pm$ 0.47
Maize	2.73 $\pm$ 0.53	39.1	3.30 $\pm$ 0.52	1.74 $\pm$ 0.25	24.9	2.95 $\pm$ 0.43
Wheat	2.58 $\pm$ 0.52	37.4	3.82 $\pm$ 0.56	2.11 $\pm$ 0.33	30.9	3.28 $\pm$ 0.47
Average (kj)/100 g body wt/24 hrs	35.44 $\pm$ 2.66	42.66 $\pm$ 5.09			36.20 $\pm$ 5.69	36.72 $\pm$ 8.68

Table 3. Relative consumption of grains (g, Mean $\pm$ S.E.) and their respective roasted forms by *T. indica*.

Baits	Av. daily intake (ADI)	Per cent consumption	Palatability rank
Bajra, grain	2.08 $\pm$ 0.52	10.82	6
Bajra, roasted grain	2.29 $\pm$ 0.36	11.91	5
Bengal gram, grain	1.75 $\pm$ 0.37	9.10	7
Bengal gram, roasted grain	2.58 $\pm$ 0.38	13.42	2
Jowar, grain	0.50 $\pm$ 0.11	2.60	10
Jowar, roasted grain	1.17 $\pm$ 0.27	6.08	9
Maize, grain	1.30 $\pm$ 0.23	6.76	8
Maize, roasted grain	2.34 $\pm$ 0.40	12.17	4
Wheat, grain	2.56 $\pm$ 0.47	13.31	3
Wheat, roasted grain	2.65 $\pm$ 0.58	13.78	1

3.6 Additives

These gerbils clearly made an easy choice for sesame oil (*Sesamum indicum*) against groundnut oil (*Arachis hypogea*) among all forms of foodgrains *viz* whole grains, cracked grains and flours (tables 4, 5 and 6).

3.7 Trial among top ranking baits

Except for wheat flour + 10% sesame oil + 1% sugar, which is expensive, all the top ranking food items were exposed together. Maize flour along with 10% sesame oil appeared to be the most preferred bait for this gerbil (table 7). No significant difference is, however, noticed between the top ranking bait (maize flour + 10% sesame oil), cracked wheat and wheat grain + 2% sesame oil which occupied 2nd and 3rd positions respectively ($t=1.53$ and 1.48 respectively). However, when locally grown grains were evaluated the preference was wheat > bajra > jowar.

3.8 Energy budget

Gerbils consumed different kinds of food in varied forms and in different combinations of oil, salt and sugar. Thus, rodents maintained their body weight at a minimum of 15.9 kJ/100g body weight/24 hr on jowar to a maximum of 86.1 kJ/100g body weight/24 hr when fed on wheat (tables 1-8).

4. Discussion

4.1 Selection of bait in no-choice and free-choice tests

No significant differences could be observed in the average daily intakes (ADI) of the same food when provided in no-choice and free-choice tests. But relatively more food was consumed in no-choice or single-choice test (table 8). Therefore, single food item should be used to bait these rodents. This will benefit in two ways (i) more poison bait will be consumed (ii) in case of exposing poison in a mixture of bait materials; due to development of baitshyness, we are left with no other preferred bait to tackle residual population as these rodents will normally reject even the individual component of the mixture on subsequent exposures. It is also observed that *Tatera* sampled every food item. This sampling led to selection of superior food, *i.e.*, wheat was preferred in 7 out of 13 tests which is also supported

Table 4. Relative food consumption (g, Mean $\pm$ S.E.) with oils by *T. indica*.

Food item	Whole grains + 2%			Cracked grains + 2%		
	Arachis oil	Energy (kj)	Sesame oil	Energy (kj)	Arachis oil	Energy (kj)
Bajra	2.79 $\pm$ 0.65	44.22	2.43 $\pm$ 0.63	38.49	1.35 $\pm$ 0.23	21.38
Barley	2.98 $\pm$ 0.46	40.09	3.05 $\pm$ 0.41	45.10	1.37 $\pm$ 0.30	23.22
Bengal gram	3.13 $\pm$ 0.57	49.45	2.84 $\pm$ 0.56	44.89	—	—
Green gram	—	—	—	—	2.52 $\pm$ 0.37	37.07
Maize	3.47 $\pm$ 0.46	52.21	3.45 $\pm$ 0.47	51.92	—	—
Moth	—	—	—	—	2.14 $\pm$ 0.31	31.12
Wheat	3.49 $\pm$ 0.49	53.09	4.01 $\pm$ 0.54	61.04	1.73 $\pm$ 0.27	26.31
Average kj/100 g body weight/24 hr		47.79 $\pm$ 5.51		48.28 $\pm$ 3.84		27.82 $\pm$ 2.84
						29.06 $\pm$ 3.06

Table 5. Relative consumption (g/100 g body weight/24 hr, Mean $\pm$ S.E.) of flours with oils, sugar and salt by *T. indica*.

Food item	Flours + 10%			Flours + 10% sesame oil + 1%		
	Arachis oil	Energy (kj)	Sesame oil	Energy (kj)	Salt	Energy (kj)
Bajra	1.85 $\pm$ 0.43	34.9	3.20 $\pm$ 0.49	60.6	1.85 $\pm$ 0.31	35.0
Barley	1.86 $\pm$ 0.41	33.2	2.05 $\pm$ 0.41	36.6	0.90 $\pm$ 0.21	26.0
Bengal gram	3.70 $\pm$ 0.49	51.7	2.93 $\pm$ 0.37	55.3	2.62 $\pm$ 0.42	49.4
Maize	3.44 $\pm$ 0.50	62.3	3.74 $\pm$ 0.47	67.8	1.97 $\pm$ 0.33	39.7
Wheat	3.44 $\pm$ 0.39	62.9	3.25 $\pm$ 0.37	59.4	3.14 $\pm$ 0.27	57.4
Average kj/100 g body weight/24 hr		49.00 $\pm$ 6.44		56.00 $\pm$ 5.25		38.72 $\pm$ 7.10
						45.14 $\pm$ 7.00

Table 6. Relative consumption of grains (g/100 g body weight/24 hr, Mean $\pm$ S.E.) with oil, salt and sugar by *T. indica*.

Food item	Whole grains + 2% arachis oil + 1%				Cracked grains + 2% sesame oil + 1%			
	Salt	Energy (kJ)	Sugar	Energy (kJ)	Salt	Energy (kJ)	Sugar	Energy (kJ)
Bajra	2.79 $\pm$ 0.92	44.22	2.62 $\pm$ 0.70	41.50	0.68 $\pm$ 0.18	10.75	1.66 $\pm$ 0.25	26.27
Barley	2.61 $\pm$ 0.39	38.74	2.97 $\pm$ 0.39	43.22	1.67 $\pm$ 0.38	24.72	1.55 $\pm$ 0.20	22.50
Bengal gram	3.27 $\pm$ 0.61	51.67	3.02 $\pm$ 0.50	47.73	—	—	—	—
Green gram	—	—	—	—	1.16 $\pm$ 0.16	17.02	1.25 $\pm$ 0.24	18.36
Maize	2.26 $\pm$ 0.30	33.97	3.24 $\pm$ 0.41	48.78	—	—	—	—
Moth	—	—	—	—	1.88 $\pm$ 0.30	27.32	1.99 $\pm$ 0.38	29.34
Wheat	5.01 $\pm$ 0.60	76.27	4.71 $\pm$ 0.56	71.67	1.08 $\pm$ 0.23	16.40	1.32 $\pm$ 0.31	20.04
Average kJ/100 g body wt/24 hr		48.96 $\pm$ 7.45		48.24 $\pm$ 4.80		19.22 $\pm$ 2.99		23.28 $\pm$ 2.00

Table 7. Relative consumption (g/100 g body weight/24 hr, Mean $\pm$ S.E.) of best ranking food items from each test.

Food items	Av. daily intake	Per cent consumption	Palatability index
Bengal gram, flour	1.51 $\pm$ 0.36	9.9	6
Green gram, cracked + 2% arachis oil	1.44 $\pm$ 0.23	8.6	8
Maize flour + 10% sesame oil	2.92 $\pm$ 0.48	17.5	1
Moth, cracked grain	0.72 $\pm$ 0.18	4.3	10
Moth, cracked grain + 2% sesame oil + 1% sugar	1.03 $\pm$ 0.22	6.2	9
Wheat, cracked grain	1.94 $\pm$ 0.43	11.6	2
Wheat, roasted grain	1.80 $\pm$ 0.39	10.8	5
Wheat, whole grain	1.67 $\pm$ 0.32	11.0	4
Wheat, whole grain + 2% arachis oil	1.94 $\pm$ 0.46	11.6	2
Wheat, whole grain + 2% arachis oil + 1% salt	1.53 $\pm$ 0.26	9.2	7

Table 8. Comparison of relative consumption of locally grown food grains in single and multiple (free) choice tests.

Food grains	ADI (g)/100 g body weight (Mean $\pm$ S.E.)				't' between a and b
	Multiple choice (a)	Energy (kJ)	Single choice (b)	Energy (kJ)	
Bajra	3.79 $\pm$ 0.62	57.20	5.38 $\pm$ 0.27	81.25	1.47 NS
Jowar	1.09 $\pm$ 0.12	16.06	2.55 $\pm$ 0.17	37.19	1.07 NS
Wheat	6.05 $\pm$ 0.80	87.54	5.94 $\pm$ 0.18	86.14	0.006 NS
Average kJ / 100g body wt / 24 hr		53.60 $\pm$ 20.78		68.19 $\pm$ 15.58	NS = Not Significant

from field observations that gerbils inflict severe pre-harvest losses to standing wheat crop (Advani *et al* 1982). Moth in two tests and green gram and bengal gram also appeared in the list of top choice food items once in these 13 tests. Thus, *T. indica* is not a selective feeder, this behaviour is quite important in two ways (i) gerbils surviving a poisoning campaign using zinc phosphide, apparently develop bait-shyness (Prakash and Jain 1971) and discriminate bait (single food or mixture as the case may be) subsequently. (ii) dietary variance is also advantageous in a desert ecosystem where no regular food supply is ensured; field rodents are bound to change over to the available food resource and gerbils have been observed even to consume insects in appreciable amounts (Prasad 1954; and Prakash 1962).

In the final test, wherein all the top ranking bait items from different tests were exposed together, gerbils preferred (i) maize flour + 10% sesame oil (ii) wheat + 2% sesame oil (iii) wheat; and (iv) bajra grains. Hence, these food items can be effectively used for baiting these rodents.

4.2 Role of additives

Addition of oils retarded the ADI of most of the foods provided except for the flours. As observed earlier, *T. indica* being omnivore tend to select the food on the basis of its nutritive or energy value. Therefore, gerbils consumed less of oil added foodgrains to regulate the energy intake (Lepkovsky 1948). Thus, there is no significant difference in the energy obtained from the consumption of more food grains (without oil) than from foodgrains consumed in low quantity (with oils). The consumption of flours did not decrease when oils are added, yet there is no significant difference in the energy intake from the flours and whole grains (tables 1 and 5 respectively). Gerbils clearly preferred sesame oil (*Sesamum indicum*) to groundnut oil (*Arachis hypogea*) with whole grains, cracked grains and flours. In the desert tract, wherever possible, sesame is grown and this is vividly reflected in the preference of this field rodent.

Gerbils categorically preferred salt with whole grains and sugar with cracked grains and flours. In case of cracked grains addition of salt and sugar retarded the intakes (even in terms of energy intake) and therefore, these additives should be used with utmost care. It will even be better not to use salt and sugar in the bait formulations as during field operation / poisoning treatments these additives go beyond control which may adversely affect the ingestion of baits.

4.3 Energy budget

Metabolic rate is positively correlated with home range (McNab 1963) and the small home range of *T. indica*, therefore, indicates low basal metabolic rate (BMR = 0.87 at 86.8 g body weight, Goyal and Ghosh 1982). However, low BMR ensures saving of pulmonary water loss (Goyal *et al* 1982) which is of considerable magnitude in hot desert environments. *T. indica* also exhibits lowest conductance value among the Indian desert rodents studied which further accounts for reduction in loss of energy. Besides this, these gerbils spend less time (22%) in foraging outside their nests which not only saves much of the energy but also avoids depredation (Goyal 1981). Thus, *T. indica* in a hot desert environment attains proper *niche* utilization by adopting to nocturnality; constructing simple burrows; low BMR mass function; low conductance mass function at a very low energy budget (Av. 35.4 to 56 kJ / 100g body weight / 24 hr) as compared to very high energy budget (246.9 to 254.5 kJ / 100 g body weight / 24 hr) required by same species (*T. indica*) in a non-desert (humid) environment (Vasanthakumari and Khan 1978).

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Juvenoid-induced supernumerary larval instars in certain stored grain insects

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Abstract. In *Ephestia cautella*, *Corcyra cephalonica* and *Tribolium castaneum*, multiple and viable supernumerary larval moults are brought about by treatment of the last larval instar with juvenoids. The normal last larval instar responds to such treatment throughout its life but the number of extra moults is age and dose-related. Supernumerary instars show progressive increase in weight, size and head width. Dyar's Law of growth is not strictly applicable. Until a certain stage, a single treatment or removal from juvenoid mixed medium is followed by 2 or 3 further moults and the insects metamorphose normally. Administration of juvenoids with food is more effective than topical treatment.

Keywords. Stored grain insects; juvenoid effect; supernumerary larval moults; *Ephestia cautella*, *Corcyra cephalonica*, *Tribolium castaneum*

1. Introduction

The production of supernumerary larval moults (SNM) by juvenoid treatment is the most categorical juvenilising effect but viable supernumerary larval instars have not been successfully produced in many cases. In insects where supernumerary moults have been produced, there may be only one or two such moults e.g. *Choristoneura fumiferana* (Retnakaran 1973); *Pectinophora gossypiella* (Cawich *et al* 1974); *Spodoptera litura* (Negishi *et al* 1976; Sunderamurthy 1976; Srivastava and Prasad 1980); *Ephestia kuhniella* (Tan 1975) etc., or several such moults e.g. *Trogoderma granarium* (Bhatnagar-Thomas 1972; Metwally and Sehna 1973; Srivastava and Srivastava 1974); *Galleria mellonella* (Plantevin 1975). In cases where repeated supernumerary moults may be brought about, several questions arise e.g. which factors are associated with such moults, what kind of treatment brings about greater response, what is the pattern of growth of supernumerary instars, if any, etc. Thus in *T. granarium* while Metwally and Sehna (1973) found that only the early part of the life of the last larval instar is sensitive to juvenoid action, Srivastava and Srivastava (1974) in the same insect and Ishaya and Yablonski (1976) in *Tribolium castaneum* reported that the period of sensitivity extends to almost the end of the life of the instar. Similarly while Bhatnagar-Thomas (1972) and Metwally and Sehna (1973) could not correlate the dose of juvenoid with the number of supernumerary moults in *T. granarium*, Srivastava and Srivastava (1974) found a direct relationship between the two.

Metwally and Sehna (1973) did not report growth in supernumerary instars but Bhatnagar-Thomas (1972), Srivastava and Srivastava (1974) and Ishaya and Yablonski (1976) noted such growth.

Thus evidently the questions have not been satisfactorily dealt with. The present study has been carried out with a view to answer these.

2. Material and methods

2.1 Rearing of insects

Larvae of the three stored grain insects *viz.* *Ephestia cautella* Walker (Lepidoptera: Phycitidae), *Corcyra cephalonica* Staint. (Lepidoptera: Phycitidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) were collected from the local grain market and reared in the laboratory at $28 \pm 1^\circ\text{C}$ in a food medium comprising corn meal, wheat flour, dried yeast, and molasses in the ratio of 60:15:5:20. A sizable stock of synchronised cultures was raised before the experiments were begun.

2.2 Juvenoids used

The following two juvenoids were used

(i) 6,7-epoxy-3, ethyl-1-(*p*-ethyl phenoxy) 7 methyl-nonane (RO,10-3108/018 of Dr R Maag Ltd., Basel, Switzerland) and (ii) Ethyl (2E,4E)-11-methoxy-3, 7,11-trimethyl-2,4 dodecadiene-thiolate (ZR-619 of Zoecon Corporation, Palo Alto, California, USA).

2.3 Method of treatment

2.3a Topical treatment: Last instar larvae of different ages of the three insects were topically treated with different quantities of the juvenoids diluted in acetone as described earlier (Srivastava and Srivastava 1982).

Treated insects were observed regularly. When the controls pupated, the treated specimens of *C.cephalonica* and *E.cautella* were taken out from the cocoon for observation while those of *T.castaneum* were observed as such.

2.3b Administration with food: Last instar larvae were transferred to food media containing different concentrations of the juvenoids as described earlier (Srivastava and Srivastava 1974; Srivastava 1980). The food hormone mixture was changed every fortnight so as to maintain a uniform concentration of the juvenoid during the period of experiment.

2.4 Determination of ED_{50} values

In order to evaluate the effectiveness of the compounds with respect to the two kinds of treatment on the three insect species, the doses at which 50% treated insects showed some kind of a response (ED_{50} values) were calculated by plotting a graph of doses *vs* probits of the total percentage of insects affected in any way by juvenoid treatment and then the antilog of the log dose at which 50% specimens were affected were determined.

2.5 Measurement of treated insects

To estimate the growth of the supernumerary instars, the insects were weighed and then killed, mounted and measured. In each case, the average of 20 individuals has been taken.

3. Observation

3.1 *Ephestia cautella*

3.1a Topical treatment: One to six day-old last instar larvae were applied 5, 10, 15 and 20 μg of the juvenoid RO, 10-3108/018. 25 larvae were administered the same dose. The treatment caused death in a few cases. Supernumerary larval moults and production of larval-pupal intermediates were observed.

It was observed (table 1) that with 5 µg per insect no death occurred but with 20 µg up to about 12% larvae died. Older larvae were more susceptible.

Even the lowest dose of 5 µg led to supernumerary moults in some larvae. However, younger the larvae and higher the dose, greater the number of larvae undergoing supernumerary moults. A single treatment may lead to 3 or 4 successive supernumerary moults and the supernumerary larvae are viable and complete the metamorphosis unless they are again treated with the juvenoid.

Larval-pupal intermediates are produced when larger doses are given to relatively older larvae. The maximum number (28%) of these is produced when 6 day-old larvae are administered 20 µg of the juvenoid. Irrespective of the extent of larval/pupal characters, these intermediates are invariably non-viable.

The ED₅₀ values for 1, 3, 4, 5 and 6 day-old larvae were 7.07, 9.12, 13.34 and 17.18 µg respectively (table 9).

3.1 b Rearing in juvenoid mixed food: Two to three day-old 6th instar larvae in groups of 100 each were transferred to food mixed with 2-500 ppm of the same juvenoid. The effects given in table 2 generally resemble that of topical treatment.

As little as 2 ppm of the juvenoid caused 9% larvae to undergo supernumerary moult or form l-p intermediates. As the concentration increases, more and more larvae undergo supernumerary moults (70% at 40 ppm and 100% at 80 ppm or more) and as many as 15-22 supernumerary moults may occur. At concentrations

Table 1. Effect of topical application of the juvenoid (RO,10-3108/018) to the last instar larvae of *E. cautella*. 25 larvae were used in each treatment.

Age (day)	Dose (µg/insect)	% l-p	% pupae	% SNM	No. of SNM	% larvae dead
1	5	0	68	32	1	0
1	10	0	28	68	1-2	4
1	15	20	0	76	1-3	4
1	20	12	0	88	2-4	0
3	5	0	84	16	1	0
3	10	0	44	56	1-2	0
3	15	24	16	60	1-3	0
3	20	16	0	76	2-4	8
4	5	8	76	12	1	4
4	10	16	52	24	1-2	8
4	15	8	28	60	1-3	4
4	20	20	8	68	2-4	4
5	5	0	100	0	0	0
5	10	24	60	16	1-2	0
5	15	8	36	52	1-3	4
5	20	12	24	56	2-4	8
6	5	0	100	0	0	0
6	10	16	76	8	1-2	0
6	15	16	52	28	1-3	4
6	20	28	32	28	1-3	12

l-p = larval-pupal intermediate; SNM = supernumerary larval moult.

Table 2. Effect of rearing 2-3 day old 7th instar larvae in the media mixed with different concentrations of the juvenoid (RO,10-3108/018). 100 larvae were used in each treatment.

Conc. (ppm)	<i>E. cautella</i>				<i>C. cephalonica</i>			
	% l-p	% pupae	% SNM	No. of SNM	% l-p	% pupae	% SNM	No. of SNM
0	0	100	0	0	0	100	0	0
2	3	91	6	1-2	8	79	13	1-3
4	4	84	12	2-4	9	66	25	3-5
6	11	69	20	2-6	9	62	29	3-6
8	16	62	22	4-8	15	44	41	4-6
10	26	45	29	4-8	26	22	52	5-8
20	36	22	42	6-10	11	11	78	6-10
30	33	13	54	8-10	0	0	100	8-12
40	25	5	70	8-12	0	0	100	10-15
50	16	0	84	10-12	0	0	100	15-20
60	9	0	91	12-15	0	0	100	15-20
70	4	0	96	14-20	0	0	100	15-22
80	0	0	100	15-22	0	0	100	15-18
90	0	0	100	15-22	0	0	100	10-15
100	0	0	100	10-16	0	0	100	10-15
200	0	0	100	8-14	0	0	100	6-10
300	0	0	100	6-12	0	0	100	6-8
500	0	0	100	6-8	0	0	100	6-8

beyond 80 ppm, the number of supernumerary moults decreases and the larvae finally die. Up to 60 ppm supernumerary moults may metamorphose or produce l-p intermediates. Even after they are removed from hormone mixed media, larvae may undergo 2-3 further moults and then pupate.

L-p intermediates are generally produced at lower concentrations (2-70 ppm). A maximum of 36% intermediates are produced with 20 ppm. Intermediates do not moult further and die whether they remain in the treated media or not. The ED_{50} value was 9.441 ppm (table 9).

3.2 *Corcyra cephalonica*

3.2 a Topical treatment: Seventh instar larvae (1-7 day old) were treated with 5, 10, 15 and 20 μ g of the juvenoid RO, 10-3108/018 in the same way as in *E. cautella*. The effect produced (table 3) was similar to that in *E. cautella*.

Application of 15 μ g of the juvenoid to 1, 3, 5, 6 and 7 day old larvae produced 2-4 supernumerary larval moults in 64, 52, 68, 64 and 44% cases, 24, 12, 32, 16 and 24% larvae produced l-p intermediates, 12, 32, 0, 20 and 32% larvae pupated normally and the rest died. The maximum number (44%) of intermediates is produced when 7 day-old larvae are treated with 20 μ g of the juvenoid. With 5 μ g intermediates are not produced and no lethal effect is seen.

The number of supernumerary larval moults is related to the amount of juvenoid applied. Thus with 5, 10, 15 and 20 μg , the number of SNM's was 1, 1-3, 2-4 and 2-5 respectively. As in the case of *E. cautella* supernumerary larvae metamorphosed normally unless a second application was made. The ED_{50} values for 1, 3, 5, 6 and 7 day old larvae were 8.913, 9.333, 7.08, 13.03 and 13.24 μg respectively (table 9).

3.2 b Rearing in juvenoid mixed food: The seventh instar larvae (2-3 day old) were transferred to food mixed with 2-500 ppm of the same juvenoid. The results (table 2) resembled those of *E. cautella*. Up to 20 ppm a smaller number of larvae underwent normal pupation (79% in 2 ppm, 11% in 20 ppm) while a greater number had supernumerary moults. The rest produced l-p intermediates, the maximum percentage (26%) being produced at 10 ppm. At 30 ppm 100% larvae had supernumerary moults. The number of supernumerary moults increased up to 60-70 ppm (15 to 22 additional moults), beyond which it declined.

As in *E. cautella*, the l-p intermediates were unable to moult or to develop further and hence died, whether they remained in the treated media or not. If supernumerary larvae were transferred from treated to untreated food up to 16 supernumerary moults, they had 1-3 further moults and then metamorphosed. The ED_{50} value was 6.607 ppm (table 9).

Table 3. Effect of topical application of the juvenoid (RO, 10-3108/018) to the last instar larvae of *C. cephalonica*. 25 larvae were used in each treatment.

Age (day)	Dose ($\mu\text{g}/\text{insect}$)	% l-p	% pupae	% SNM	No. of SNM	% larvae dead
1	5	0	88	12	1	0
1	10	16	40	44	1-3	0
1	15	24	12	64	2-4	0
1	20	0	0	100	2-5	0
3	5	0	88	12	1	0
3	10	20	32	36	1-3	12
3	15	12	32	52	2-4	4
3	20	0	0	100	2-5	0
5	5	0	96	0	0	4
5	10	28	32	28	1-3	12
5	15	32	0	68	2-4	0
5	20	8	0	80	2-5	12
6	5	0	100	0	0	0
6	10	0	52	40	1-3	8
6	15	16	20	64	2-4	0
6	20	28	0	72	2-5	0
7	5	0	100	0	0	0
7	10	24	56	16	1-3	4
7	15	24	32	44	2-4	0
7	20	44	4	44	2-5	8

Table 4. Effect of topical application of the juvenoid (ZR- 619) to the last instar larvae of *T. castaneum*. 30 larvae were used in each treatment.

Age (day)	Dose ($\mu\text{g}/\text{insect}$)	% l-p	% pupae	% SNM	No. of SNM
1	0.05	7	56	37	1-2
1	0.1	3	47	50	1-2
1	0.5	13	33	54	1-2
1	1.0	0	37	63	1-3
1	1.5	0	27	73	1-3
2	0.05	0	50	50	1-2
2	0.1	13	44	43	1-2
2	0.5	20	33	47	1-3
2	1.0	10	27	63	1-3
2	1.5	3	30	67	1-3
3	0.05	13	60	27	1-2
3	0.1	20	40	40	1-2
3	0.5	17	50	33	1-3
3	1.0	17	36	47	1-3
3	1.5	24	33	43	1-3
4	0.05	20	47	33	1-2
4	0.1	27	36	37	1-2
4	0.5	40	30	30	1-3
4	1.0	30	30	40	1-3
4	1.5	40	27	33	1-3
Pre-pupae	0.5	0	100	0	0
"	1.0	0	100	0	0
"	1.5	0	100	0	0

3.3 *Tribolium castaneum*

3.3 a Topical treatment: The last instar larvae (1-4 day old) and prepupae were treated with 0.05, 0.1, 0.5, 1.0 and 1.5 μg of the juvenoid ZR-619, 30 larvae with each dose. The effect (table 4) is strikingly similar to those on *C. cephalonica* and *E. cautella* except that in this case a few winged larvae were produced.

Application of 0.05, 0.1, 0.5, 1 and 1.5 μg of the juvenoid to 4 day old larvae caused 33, 37, 30, 40 and 33% larvae to undergo 1-3 supernumerary larval moults and 20, 27, 40, 30 and 40% larvae produced l-p intermediates respectively. With the highest dose (1.5 μg) administered to 1, 2, 3 and 4 day old larvae, supernumerary moults occurred in 73, 67, 43 and 33% larvae respectively and 0, 3, 24 and 40% larvae produced l-p intermediates. Thus more larvae undergo supernumerary moults when they are young and treated with higher doses, whereas treatment of older larvae induces production of l-p intermediates. Treatment of prepupae even with the highest dose did not affect pupation but these pupae did not emerge at all. The ED_{50} values for 1, 2, 3 and 4 day old larvae were 0.066, 0.05, 0.075 and 0.04 μg respectively (table 9).

3.3 b Rearing in juvenoid mixed food: The 6th instar larvae (1-2 day old) were transferred to food mixed with 2 to 100 ppm of the same juvenoid (ZR-619) and their fate observed (table 5).

Even with 2 ppm 14% larvae formed l-p intermediates and as the concentration increased, the percentage of intermediates increased till with 8 ppm a maximum of 36% intermediates were obtained. Further increase in the concentration increased the number of larvae undergoing supernumerary moults and with 50 ppm or more 100% larvae had supernumerary larval moults. The maximum number (18) of supernumerary moults was obtained with 50 ppm. Further increase in the concentration of the juvenoid reduced the number of supernumerary moults and led to the early death of larvae in 8-12 days. However, if removed from treated to untreated food till the 10 supernumerary moult stage they underwent 1-2 further moults and then metamorphosed normally. The ED₅₀ value was 5.885 ppm (table 9).

3.4 Study of supernumerary instars

3.4 a Weight and size of supernumerary larval instars, their pupae and adults :

Table 6 gives the average weight of the normal last instar larva, pupa and adult as well as the weight of the supernumerary larval instars and pupae and adults derived from the superlarvae. Similarly table 7 gives the average size (l × b) of these insects.

Table 5. Effect of rearing 12 days old 6th instar larvae of *T. castaneum* in the media mixed with different concentrations of the juvenoid (ZR-619). 100 larvae were used in each treatment.

Conc. (ppm)	% l-p	% pupae	% SNM	No. of SNM
0	0	100	0	0
2	14	78	8	1
4	22	64	14	1-2
6	26	48	26	1-3
8	36	18	46	2-4
10	23	10	67	4-6
20	11	4	85	4-8
30	4	8	88	8-11
40	2	0	98	10-15
50	0	0	100	10-18
60	0	0	100	10-13
70	0	0	100	8-10
80	0	0	100	6-8
90	0	0	100	4-5
100	0	0	100	2-3

Table 6. Average weight (mg) of different supernumerary larvae and their pupae and adults. Average of 20 insects.

No. of s.n. larval instars	<i>E. cautella</i>			<i>C. cephalonica</i>			<i>T. castaneum</i>		
	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult
0 (normal last instar)	14.1	13.5	11.8	16.8	17.1	15.3	2.5	2.8	2.7
2	16.0	15.4	13.6	19.4	19.3	17.4	3.3	3.3	3.3
4	19.5	19.7	17.2	22.9	23.3	21.8	3.9	4.0	3.8
8	26.8	26.4	24.2	31.0	31.2	30.2	5.3	5.5	5.4
12	34.6	33.7	30.3	41.0	41.7	38.4	5.0	5.0	5.1
16	41.2	40.3	37.4	48.5	47.5	45.2	4.2	—	—
20	38.5	39.2	36.3	47.5	46.4	42.5	—	—	—

Table 7. Average length and width (mm) of different supernumerary larvae and their pupae and adults. Average of 20 insects.

No. of s.n. larval instars	Larva		Pupa		Adult	
	L	W	L	W	L	Wing expanse
<i>E. cautella</i>						
0	13.5	2.0	6.5	1.7	6.8	10.6
2	14.4	2.5	8.0	1.9	7.0	11.2
4	15.6	2.7	8.5	1.9	7.4	11.8
8	16.8	3.2	11.0	2.2	8.2	12.4
12	18.2	4.0	11.5	2.6	9.2	12.9
16	19.5	4.2	12.1	3.0	10.0	13.7
20	21.0	4.6	12.5	3.5	10.3	14.2
<i>C. cephalonica</i>						
0	13.0	2.0	7.5	1.7	8.2	11.3
2	13.5	2.5	8.0	2.0	9.0	12.3
4	14.0	3.1	9.5	2.5	9.8	13.6
8	15.5	3.9	11.5	3.2	10.2	15.4
12	17.0	4.5	12.1	3.8	10.8	17.1
16	18.5	4.8	12.5	4.0	11.3	18.5
20	20.0	4.9	13.0	4.2	11.5	18.5
<i>T. castaneum</i>						
0	5.2	1.0	3.0	1.0	3.5	1.4
2	6.4	1.0	3.3	1.2	3.8	1.5
4	6.8	1.0	3.8	1.3	4.1	1.5
8	8.4	2.0	4.5	1.4	4.6	1.7
12	7.5	2.0	4.7	1.4	4.7	1.6
16	6.1	2.0	—	—	—	—

L=length; W=width; 0=Normal last instar

It will be seen that as the larva passes through successive supernumerary larval moult it grows in size and weight. Thus the 16th supernumerary larval instar of *E. cautella* measures 19.5×4.25 mm and weighs 41.25 mg as compared to 13.5×2 mm and 14.1 mg of the last larval instar *i.e.* the 16th supernumerary larval instar grows about 3 times in weight.

Similarly the pupa and adult produced from the 16th supernumerary instar larva measure 12.15×3 mm and 10×13.7 mm (wing expanse) and weigh 40.3 mg and 37.4 mg respectively against the normal pupa and adult measuring 6.5×1.7 mm and 6.8×10.6 mm and weighing 13.5 mg and 11.86 mg respectively.

C. cephalonica and *T. castaneum* also show a similar pattern of growth in their size and weight in the successive supernumerary moults but the 16th supernumerary larval instar of *C. cephalonica* shows more than 3 times increase in body weight while the 8th supernumerary instar larva of *T. castaneum* shows more than 2 times increase in body weight.

3.4 b Width of head capsule of normal and supernumerary larval instars: The average head capsule width of different normal and supernumerary larval instars

has been presented in table 8. With successive supernumerary moults, the width of head capsule increases progressively with increase in weight and size.

The average rate of increase in the normal larval instars of *E. cautella*, *C. cephalonica* and *T. castaneum* is 1.408, 1.356 and 1.318 times respectively whereas in the supernumerary larval instars it is 1.064, 1.054 and 1.012 times only. Thus Dyar's Law is not strictly applicable in the supernumerary instars of all the three species.

4. Discussion

Apart from lethal effect, exogenous treatment of insect larvae with juvenile hormone or its analogues has been reported to cause 4 distinct kinds of effects (i) supernumerary larval moults, (ii) production of larval-pupal intermediate forms, (iii) ecdysial failure and (iv) prolongation of life of treated instar frequently followed by death. Of these, the three stored grain insects used in the present study show supernumerary larval moults and production of larval-pupal intermediates.

All insect larvae do not undergo a supernumerary moult as a result of juvenoid treatment; e.g. *Sarcophaga bullata* (Srivastava and Gilbert 1969); *Caryedon gonagra* (Metwally and Sehnal 1973); *Dermestes vulpinus* and *Leptinotarsa* sp. (Slama 1974); *Hyalophora cecropia* (Riddiford 1972); *Bombyx mori* (Akai and Kobayashi 1971); *Heliothes irrescens*, (Benskin and Vinson 1973); *Cydia* (*Car-*

Table 8. Width of head capsule (in μ) of normal and supernumerary larval instars. Average of 20 insects.

	<i>E. cautella</i>		<i>C. cephalonica</i>		<i>T. castaneum</i>	
No. of larval instars	Average head width	Predicted mean by Dyar's Law	Average head width	Predicted mean by Dyar's Law	Average head width	Predicted mean by Dyar's Law
Normal						
1	175	—	184	—	175	—
2	242	245	240	257	225	245
3	412	338	328	336	295	315
4	604	576	464	459	420	473
5	828	845	636	649	600	688
6	980	1160	874	890	795	840
7	1005	1372	1080	1223	—	—
Average rate of increase	1.408		1.356		1.318	
Supernumerary						
0	1005	—	1080	—	795	—
2	1072	1459	1125	1543	825	1155
4	1125	1606	1185	1722	885	1239
8	1237	1764	1387	2016	945	1323
12	1342	1911	1417	2065	1072	1501
16	1470	2121	1620	2299	1200	1680
20	1620	2310	1800	2583	—	—
Average rate of increase	1.064		1.054		1.012	

pocapsa pomonella, (Gelbic and Sehnal 1973). In others a single, or rarely two such moults may take place but the supernumerary instars are usually non-viable and do not start or complete metamorphosis e.g. *C. fumiferana* (Retnakaran 1973); *Pieris brassicae*, (Benz 1973); *S. litura* (Negishi *et al* 1976; Sunderamurthy 1976; Srivastava and Prasad 1980); *E. kuhniella* (Tan 1975); *Cerura vinula* and *Sphinx ligustri* (Hintz-Podufal 1975); *Pyrrhocoris apterus* (Slama and Williams 1965); *Dysdercus cingulatus* (Saxena and Williams 1966; Babu and Slama 1972); *Rhodnius prolixus* (Wigglesworth 1969); *Oncopeltus fasciatus* (Riddiford 1970); *Bovicola limbata* (Chamberlain and Hopkins 1970); *Graphosoma italicum* and *Triatoma infestans* (Slama 1974); *Locusta migratoria* (Roussel 1975); *Earias fabia*, (Srivastava and Srivastava 1981); *Papilio demoleus* (Srivastava and Prasad 1982) etc. In a few cases, repeated viable, supernumerary larval moults take place e.g. *T. granarium* (Bhatnagar-Thomas 1972; Metwally and Sehnal 1973; Srivastava and Srivastava 1974); *G. mello-nella* (Plantevin 1975). The three insects studied here belong to this category. Indeed they may undergo more than 15 moults and except in the last few instars they feed normally, complete metamorphosis and produce giant pupae and adults.

One of the reasons for absence of supernumerary moults or their inviability is the suppression or interference in the process of moulting which is evidently caused when juvenoid treatment somehow interferes with the secretion, release or action of the moulting hormone. When interference is partial the process is described as ecdysial failure. Conversely in *Trogoderma*, *Galleria* and the three insects studied here the treatment does not affect the ecdysial process and in contrast to most insects the superlarvae feed, grow, moult and metamorphose normally. In higher Diptera (Srivastava and Gilbert 1969) and other insects, e.g. *B. mori*, (Akai and Kobayashi 1971); *H. cecropia*, (Riddiford 1972) and *C. pomonella*, (Gelbic and Sehnal 1973) where juvenoid treatment prolongs instar life without moulting, it has been suggested that moulting hormone is completely

Table 9. ED₅₀ for larvae of the 3 insect species.

Name of insect	Juvenoid	Age of larvae (day)	Oral administration (ppm)	topical treatment (µg/insect)
<i>E. cautella</i>	RO,10-3108/018	1	—	7.079
		3	9.441	9.120
		4	—	13.34
		5	—	13.55
		6	—	17.18
<i>C. cephalonica</i>	RO,10-3108/018	1	—	8.913
		3	6.607	9.333
		5	—	7.079
		6	—	13.03
		7	—	13.24
<i>T. castaneum</i>	ZR-619	1	—	0.066
		2	5.885	0.05
		3	—	0.075
		4	—	0.04

suppressed and there is not even partial ecdysis.

Srivastava *et al* (1979) suggested that the difference in the response of insects to juvenoid treatment is related to the difference in their habitat and that insects which live in a relatively constant environment with continuous food supply tend to have repeated, viable supernumerary moults. The present observations provide additional support to this contention.

In the present test insects, a single topical application of juvenoid to the last larval instar may result in 2-3 supernumerary larval moults. Edwards and Rowlands (1977) have shown that in *T. castaneum* after each normal moult, juvenile hormone is metabolised. It has also been demonstrated that the half life of the juvenile hormone in the insect body is only about 30 min (Nijhout 1975; Kramer *et al* 1977). The occurrence of two or three supernumerary moults by a single application is evidently due to an adequate supply of the juvenoid being available and indicates that a sufficient amount of the exogenously administered hormone is stored during the life of the stadium in some form and released during the next moult; or the hormone stimulates the corpora allata to remain active during the next 2 or 3 moults instead of switching off, as may normally be expected.

Bhatnagar-Thomas (1972) and Metwally and Sehnal (1973) in *T. granarium* and Plantevin (1975) in *G. mellonella* have also noted the occurrence of several supernumerary larval moults. In *T. granarium*, Srivastava and Srivastava (1974) have noted 23 and 19 supernumerary moults in female and male respectively. Both *E. cautella* and *C. cephalonica* undergo about 22 supernumerary moults each and *T. castaneum* undergoes 18 supernumerary moults. Further, it is noted that as the supernumerary instars continue to grow, the competence of the larvae to pupate is maintained, but when growth stops, supernumerary larval instars actually start attenuating, their competence to metamorphose is impaired and finally they die without pupating.

Cawich *et al* (1974) and Sunderamurthy (1976) suggested that supernumerary moults can be produced only with larger quantities of the juvenoids. We have noted a direct relationship between the quantity of hormone and the occurrence of supernumerary moults. For example whereas the threshold dose for production of supernumerary moults in *C. cephalonica* is only 2 ppm (table 2) of the juvenoid (RO, 10-3108/018) in food when 13% larvae undergo supernumerary moults, but with 40 ppm 100% larvae show this phenomenon. Similarly in topical treatment, the threshold dose for production of such moults is only 5 µg of the same juvenoid when 12% of 1 to 3-days old larvae show this effect, whereas 100% larvae of the same age show supernumerary moults with 20 µg of the juvenoid (table 1).

Metwally and Sehnal (1973) in *T. granarium*, Retnakaran (1973) in *C. fumiferana* and Tan (1975) in *E. kuhniella* found the early part of the last larval instar to be sensitive to juvenoid treatment. Abdallah *et al* (1974) in *S. littoralis* and Hintz-Podufal (1975) in *C. vinula* reported that the middle part of the last larval instar shows maximum sensitivity while Sehnal *et al* (1976) in *S. littoralis* observed that the later part of the feeding stage of the last larval instar is most sensitive. In *T. castaneum*, Ishaya and Yablonski (1976) found that the entire period from the very beginning of the life of the last larval instar till pupation is sensitive to juvenoid. Our observation agrees with that of Ishaya and Yablonski and in the 3 stored grain insects studied here, treatment with relatively larger quantities of the juvenoid even on the last day of the larval life causes supernumerary moults in some larvae. However, apparently the earlier part of the life of the instar seems to be the optimum period for the production of supernumerary

moult. Treatment of 1-3 day old larvae of *C. cephalonica* (life span of last instar 8 days) gives supernumerary moults in 100% cases with 20 µg and in *E. cautella* (life span of last instar 7 days) treatment of 1-day old larvae with 20 µg gives supernumerary moults in 88% cases. In *T. castaneum*, the period of maximum sensitivity lies in the first two days (total life span of the last instar 4 days).

Apparently in the present insects pupal transformation does not irreversibly begin in any part of the body until very late and thus there is no clear critical period as in some other insects.

4.1 Study of supernumerary larval instars

Retnakaran (1973) in *C. fumiferana*, Hintz-Podufal (1975) in *C. vinula*, Plantevin (1975) in *G. mellonella*, Sunderamurthy (1976) in *S. litura* observed an increase in size and weight of the supernumerary instars. In *T. granarium* while Bhatnagar-Thomas (1972) reported an increase in size and weight of the supernumerary larvae, Metwally and Sehna (1973) did not; Srivastava and Srivastava (1974) and Srivastava (1980) noted about 3 times and 2 times than normal increase in the weight of 14th (in female) and 10th (in male) supernumerary instar larvae. In *E. kuhniella*, Tan (1975) observed about 3 times increase in body weight while in *T. castaneum*, Ishaya and Yablonski (1976) reported 2 times increase in body weight. These workers, however, did not indicate the number of additional moults after which this extent of growth occurred.

In the three species studied here larval growth continues through the supernumerary moults until a certain maximum is reached in the 16th supernumerary instar in *E. cautella* and *C. cephalonica* (about 3 times) and in the 8th supernumerary instar in *T. castaneum* (more than 2 times). Thus our results conform with those of Tan (1975) and Ishaya and Yablonski (1976) in *E. kuhniella* and *T. castaneum* respectively. We also find that the increase in weight of supernumerary instars occurs in geometrical progression but the rate of growth of the supernumerary instars is lower than that of the normal instars.

Retnakaran (1973) studied the increase in head capsule width of 1st and 2nd supernumerary instars of *C. fumiferana* and found that it satisfied the Dyar's Law. Srivastava and Srivastava (1974) also noted an increase in head capsule width in the supernumerary instars of *T. granarium* but it did not conform to Dyar's law. In the present insects also while there is steady increase in the head width in the supernumerary instars, the rate of increase is lesser than that during the normal development.

After a certain number of supernumerary moults, the supernumerary larvae do not grow any more and show signs of decadence in all the three cases. As they moult further they shrink and finally die without pupating. It seems that the larvae start ageing after a certain number of moults.

Unlike in *S. bullata* (Srivastava and Gilbert 1969); *B. mori* (Akai and Kobayashi 1971); *H. cecropia* (Riddiford 1972); *C. pomonella* (Gelbic and Sehna 1973); etc. these 3 insects do not show prolongation of instar life after juvenoid treatment nor do they show ecdysial failure, which again seems to be due to the fact that juvenoid treatment does not interfere at all with moulting hormone action.

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Biochemical correlates of the mechanism of salt uptake and excretion in the gills of freshwater field crab on adaptation to higher salinity

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Abstract. Male adult paddy field crabs (*Oziotelphusa senex senex*) were adapted to salinity of 34‰. After 3 months the gill lamella of the crab was sectioned and examined under an electron microscope and compared with that of freshwater crabs. The apical region nearest to cuticular boundary showed numerous foldings projecting into the cytoplasm of the epithelium. This brush border appearance disappeared in salinity-adapted crabs. Mitochondria also reduced in number in the sections of salinity adapted crabs. In seawater medium the crabs showed a hypo-osmotic regulation. This regulation is said to be involved in active sodium excretion through gills, in contrast to the active Na^+ uptake found in freshwater crabs. The ultrastructural changes have been correlated with significant reduction in cholesterol and phospholipid molar ratios of the gill as well as $\text{Na}^+ + \text{K}^+$ ATPase and divalent ion ATPase activities of particulate system of the crab. The univalent ion ATPase activity served as a marker to show the reduced uptake of Na^+ on adaptation to higher salinity. The reduced Ca^{2+} ATPase activity in the gills of seawater adapted crabs has been correlated with the reduced phospholipid content of the gill tissue.

Keywords. Salinity adaptation; lamellar epithelium; marker enzyme; ultrastructure; field crab; *Oziotelphusa senex senex*.

1. Introduction

It is now clearly understood that freshwater crustaceans actively take up salt from the medium to compensate for the loss through excretion, and this process obviously necessitates a high metabolic demand (Ramamurthy 1965). Euryhaline freshwater crustaceans on experimental transfer to hypersalinity gain salt into the hemolymph and transfer the salt load to the medium like any other euryhaline fish (Gilles 1975; Hoar 1972). Gills seem to be the principal organs of this salt uptake as well as this salt excretion depending on the salinity of the environment (Bielawski 1971). Studies carried out so far have demonstrated changes in the enzyme activities involved in the permeability properties of the membrane (Kamiya and Utida 1969; Towle *et al* 1976, 1977 with reference to the salinity adaptations. Individually many authors reported ultrastructural changes in gills following salinity adaptation of some thermoconformers (Copeland 1963; Kinne 1958). However, no correlation has been attempted between the biochemical parameters and the ultrastructural changes. This report illustrates the correlation between the permeability markers like cholesterol phospholipid ratios and $\text{Na}^+ + \text{K}^+$ ATPase activity, and ultrastructure of the gill epithelial lining in a euryhaline freshwater field crab.

2. Materials and methods

2.1. Experimental animals

The adult freshwater paddy field crabs, *Oziotelphusa senex senex* (13-14 g size) were collected from the fields in Hasserghatta near Bangalore and stocked in laboratory aquaria. Only male specimens were used for experiments. One group was directly transferred and acclimated to artificial sea water of 34‰ (Lyman and Flemming 1949) for 3 months (Subramanyam and Krishnamoorthy 1979). Another group, serving as controls was maintained in dechlorinated freshwater. Both groups were fed *ad lib* on chopped earthworms and frog muscles every other day and maintained at $27 \pm 2^\circ\text{C}$. On alternate days the media in the aquaria were also changed.

2.2. Blood ionic composition

Haemolymph of the crab was drawn into a hypodermic syringe which was introduced into the sinus of the ambulatory leg through the arthrodial membrane. Haemolymph was diluted with distilled water for measurements of Ca^{2+} , Mg^{2+} , K^+ and Na^+ by flame photometry. Chloride and sulphate were measured according to the method of Robertson and Webb (1939).

2.3. Electron microscopic studies

The gills were excised, minced with a razor blade and fixed in cold 1% glutaraldehyde in 50 mM Na-cacodylate buffer, pH 7.2, for 2 hr at 4°C . The slices were rinsed thoroughly with cacodylate buffer and post-fixed in 1% osmium tetroxide for 1 hr at room temperature ($27 \pm 2^\circ\text{C}$), dehydrated in various acetone solutions and embedded in epoxy resin. Thin serial sections (600°A) were cut with a glass knife on LKB ultramicrotome and stained with uranyl acetate and lead citrate. The sections were examined in Hitachi HUXI electron microscope at voltage 75 V.

2.4. Preparation of mitochondria and microsomes

The gills were excised, rinsed in homogenizing medium containing 0.25 M sucrose, 6 mM EDTA, 20 mM imidazole-HCl, pH 6.8, briefly blotted and homogenized in ten volumes of the same medium containing 0.1% (w/v) sodium deoxycholate (Hendler *et al* 1976). A glass homogenizer (Ootter-Elvehjem type) was utilized to prepare crude homogenates by 20 complete strokes at 1800 rev/min. The crude homogenate was filtered through double thickness cheese cloth and centrifuged at 600 g max for 30 min. The supernatant was then centrifuged at 15,000 g max for 40 min to obtain mitochondria. The pellet was suspended in 5 ml buffered medium and frozen at -15°C until analysis. The supernatant was decanted and centrifuged at 105,000 g max for 60 min (Towle *et al* 1977). After removing the supernatant by aspiration, the microsomal pellet was resuspended in 5 ml of homogenizing medium. The suspension was then gently homogenized by hand in a glass homogenizer, and frozen at -15°C until use. Post-microsomal supernatant was also collected to estimate the residual ATPase activity. This supernatant is referred to as non-particulate cell system.

2.5. Assay of $\text{Na}^+ + \text{K}^+$ ATPase activity

After thawing the mitochondrial or microsomal suspensions in ice-water, triplicate samples (containing about 20 μg protein) were preincubated for 10 min at 25°C in the

total assay medium which contained 20 mM imidazole-HCl (pH 7.8), 100 mM NaCl, 30 mM KCl and 5 mM $MgCl_2$ (final concentrations). Identical samples were preincubated in the control assay medium which contained 20 mM imidazole-HCl (pH 7.8), 130 mM NaCl, 5 mM $MgCl_2$, and 1 mM ouabain (Towle *et al* 1971). The reaction was started by adding 0.2 ml of a solution of 50 mM disodium ATP (neutralized with imidazole buffer) resulting in a final volume 2 ml. The mixtures were incubated at 25° C for 30 min; the reaction was stopped by adding 2 ml of ice-cold 10% (w/v) trichloroacetic acid. After centrifugation at 10,800 g max for 10 min, inorganic phosphate in the supernatant was estimated according to Fiske and Subba Row (1925). The activity was expressed as μ mol pi released/min/mg protein. Protein concentrations were determined according to Lowry *et al* (1951).

2.6. Assay of Ca^{2+} and Mg^{2+} ATPase activities

The divalent ion stimulated ATPase activities in the suspensions were estimated according to MacLennan (1970). The incubating mixture consisted of 1 ml suspension (0.1 mg protein; denatured in controls) in 1% bovine serum albumin (Biochemicals Unit, V P Chest Institute, Delhi) with a mixture of solution containing 2 ml 0.1 M Tris malate buffer, pH 7.3, 0.5 ml 0.05M divalent ion ($CaCl_2$ or $MgCl_2$) and 0.05M ATP.

2.7. Phospholipid and cholesterol

Total lipid from the gills was extracted in 19 volumes of ice-cold chloroform-methanol (2:1, v/v) containing butylated hydroxy-toluene (5 mg/ml). After heating at 50° C for 5 min, the suspensions were cooled to room temperature (Leas and Stanley 1957), dried under reduced pressure, and stored in a solution of chloroform-methanol (1:1, v/v). Phospholipid fraction was isolated through silicic acid column chromatography. The solvent from 0.5 ml of the fraction was evaporated and the phosphorus content of the residue was estimated according to Barlett (1959). Phospholipid content was expressed in μ atoms of P per unit weight of gill protein. Cholesterol was extracted with acetone-alcohol (3:1), saponified, obtained as digitonide and estimated according to Sperry and Webb (1959).

3. Results

Figure 1 illustrates the changes in the apical membrane of the lamellar lining in the crab gill on adaptation to higher salinity. In freshwater crabs, the apical region nearest cuticular boundary membranes are thrown into folds giving a brush border appearance. These folds penetrate deep into the cells. Dispersed fairly in the cytoplasm, the mitochondria are packed in the lamellar cells of freshwater crab. The cuticle was thick in freshwater crabs and probably got hardened due to salinity stress (figure 1).

Table 1 presents the ionic composition of the haemolymph of freshwater and salinity-adapted male crabs. Na^+ , K^+ , Ca^{2+} , Cl^- , SO_4^{2-} concentrations increased significantly on salinity adaptation. Mg^{2+} concentration did not vary (table 1). The seawater to which the crabs were adapted had 470 mM/l sodium, 58 mM/l maganesium, 10 mM/l calcium, 99 mM/l potassium, 0.156 mM/l strontium, 548 mM/l chloride and 28 mM/l sulphate (Lyman and Flemming 1940). A comparison of molar concentrations of these ions with those of blood ions indicated that the haemolymph of salinity-adapted crabs is not isotonic to the medium. Obviously the

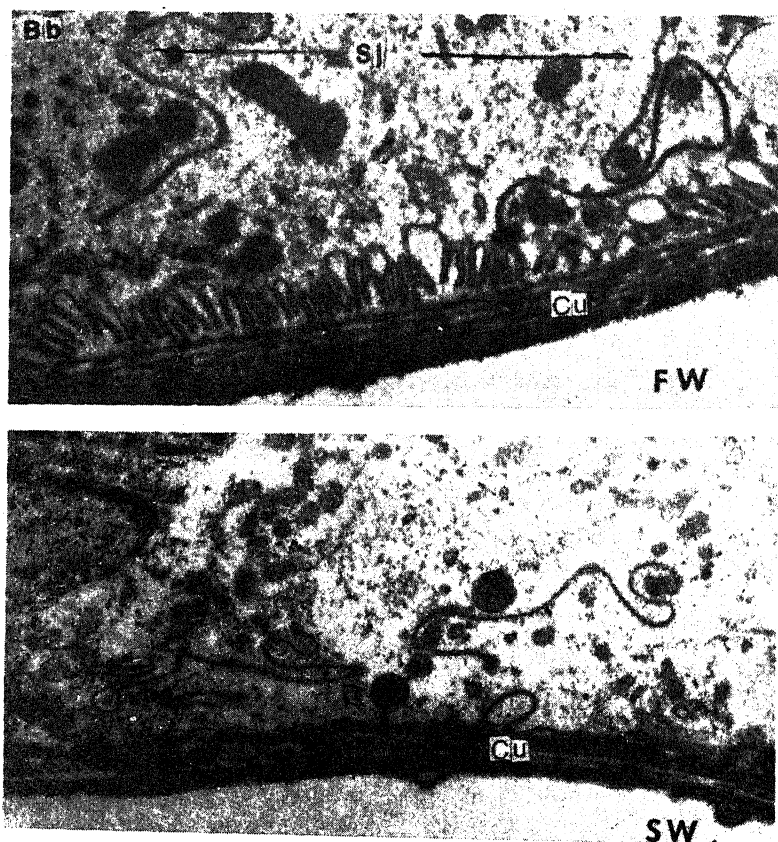


Figure 1. Electron micrograph ($\times 22,000$) of the section passing through the proximal lamellar epithelium of the gill of the crab, *Ozioelphusa senex senex*.

Abbreviations: FW, freshwater crab; SW, Seawater-adapted crab; Bb, basal boundary; Sij, systate junction; Cu, Cuticle.

Table 1. Changes in the haemolymph ionic composition of the male crab (13-14 g size) *Ozioelphusa senex senex*, on adaptation to sea water.

Ion	* mM/l haemolymph		Incidence of change on adaptation	
	Freshwater crabs	Seawater-adapted crabs		
Sodium	286 $\pm$ 21	365 $\pm$ 28	t = 5.181;	increase
Potassium	8.4 $\pm$ 0.09	12.2 $\pm$ 0.8	t = 9.939;	increase
Calcium	11.2 $\pm$ 0.26	12.9 $\pm$ 0.71	t = 3.069;	increase
Magnesium	19.8 $\pm$ 4.5	22.0 $\pm$ 5.7	t = 1.225;	no change
Chloride	238 $\pm$ 27	363 $\pm$ 19	t = 10.708;	increase
Sulphate	13.8 $\pm$ 2.1	17.4 $\pm$ 3.1	t = 2.807;	increase

* mean, $\pm$ SD of 8 observations; t = Student 't' test of statistical analysis

Table 2. Changes in the cholesterol and total phospholipid contents in the gills of crab, *Oziotelphusa senex senex* on adaptation to sea water.

Constituent	No. of observations	Mean $\pm$ SD	
		Freshwater crabs	Seawater adapted crabs
Cholesterol μ mol/ 100 mg protein	14	82 $\pm$ 14	136 $\pm$ 18
Total phospholipids μ atom P/ 100 mg protein	12	95 $\pm$ 16	38 $\pm$ 9
C:P ratio	12	0.86 $\pm$ 0.12	3.57 $\pm$ 0.18

Table 3. Changes in the $\text{Na}^+ + \text{K}^+$ ATPase activities in the gill of the crab, *Oziotelphusa senex senex* adapted to sea water.

Source	(X $\pm$ SD) mmol Pi/mg protein/min		Incidence of change on adaptation
	Freshwater crabs	Seawater-adapted crabs	
Dialyzed crude homogenate	11 $\pm$ 2.1 *	7 $\pm$ 3.2	t = 2.559; decrease
Mitochondria	23 $\pm$ 1.6	11 $\pm$ 2.5	t = 9.903; decrease
Microsomes	24 $\pm$ 1.8	7 $\pm$ 1.7	t = 16.983; decrease
Post-microsomal supernatant	13 $\pm$ 1.3	6 $\pm$ 1.2	t = 8.012; decrease

* mean $\pm$ SD of 6 observations.

haemolymph is hypertonic to the medium in freshwater crabs, and hypotonic to the medium in salinity-adapted crabs.

Table 2 summarizes the data on cholesterol and phospholipid contents of the gill of crab with reference to salinity adaptation. The cholesterol level with reference to unit protein increased in the gill following salinity adaptation in contrast to the total phospholipid level. Consequently, the molar ratios of cholesterol to phospholipid increased four-fold on adaptation to higher salinity.

The changes in ouabain sensitive $\text{Na}^+ + \text{K}^+$ ATPase activities in the particulate and non-particulate fractions of the gill of crabs with reference to salinity adaptation are presented in table 3. These data obviously show that the salinity stress has immense influence over the ATPase activity of the gill. Evidently, the particulate system shows a higher ATPase activity, in general, in the gill. On adaptation, to salinity, a significant reduction in the activity was found in all fractions.

Table 4 demonstrates the changes in divalent ion simulated ATPase activities in the gill tissue on adaptation to sea water. Divalent ion activated ATPase activities are higher in mitochondria than in microsomes. Ca^{2+} stimulation of ATPase activity is higher than that of Mg^{2+} stimulated activities. Mg^{2+} stimulated activities of mitochondrial and microsomal fractions reduced due to salinity adaptation. It is only the Ca^{2+} ATPase that showed a significant reduction in adaptation to salinity. Salinity-adapted crabs showed reduced Ca^{2+} ATPase activities significantly. Mitochondria showed a 50% reduction and microsomes nearly 30% reduction in Ca^{2+} activities. Both divalent ion activities have been found to be sensitive to sodium azide, and insensitive to ouabain.

Table 4. Changes in Ca^{2+} and Mg^{2+} ATPase activities in the gill of the crab, *Oziotelphusa senex senex* adapted to sea water.

Source	(X $\pm$ SD) mmol Pi/mg protein/min		Incidence of change on adaptation	
	Freshwater crabs	Seawater-adapted crabs		
Dialyzed homogenate				
Ca^{2+} stimulated	3 $\pm$ 1.6	2 $\pm$ 0.9	t = 1.335;	no change
Mg^{2+} stimulated	2 $\pm$ 0.91	1.8 $\pm$ 0.16	t = 0.522;	no change
Mitochondria				
Ca^{2+} stimulated	6 $\pm$ 0.28	3 $\pm$ 0.38	t = 14.159;	decrease
Mg^{2+} stimulated	2 $\pm$ 0.91	1 $\pm$ 0.79	t = 2.029;	decrease
Microsomes				
Ca^{2+} stimulated	3 $\pm$ 0.42	2 $\pm$ 0.31	t = 4.692;	decrease
Mg^{2+} stimulated	0.9 $\pm$ 0.16	0.8 $\pm$ 0.27	t = 0.475;	no change
Post-microsomal				
Ca^{2+} stimulated	4 $\pm$ 0.16	3 $\pm$ 0.19	t = 2.679;	decrease
Mg^{2+} stimulated	3 $\pm$ 0.21	2 $\pm$ 0.09	t = 10.721;	decrease

* mean $\pm$ SD of 8 observations.

4. Discussion

Oziotelphusa senex senex is an inhabitant of paddy fields in peninsular India and seems to be a remarkable species among crabs. They maintain a blood ionic balance equivalent to that of 50-55‰ seawater, and in that salinity they show a very low oxygen consumption (Krishnamoorthy and Srihari 1973). On either side of this salinity the oxygen consumption increases exhibiting a parabolic curve to increasing salinities (Krishnamachari 1980) minimum oxygen consumption prevailing in salinities equivalent to that of 50-55‰ sea water. However, the oxygen consumption in 100‰ seawater is two to three times less than that in freshwater (Krishnamoorthy and Srihari 1973).

Copeland (1968) suggested a fine structural basis for the function of active salt absorption by the gills in blue estuarine crab, *Callinectes sapidus* and the land crab, *Gecarcinus lateralis*. In these salt absorbing tissues is found a highly interdigitate epithelium within the respiratory lamellae of the gills. The brush border epithelium noted (figure 1) refers to the interdigitated epithelium described by Copeland (1968). Other cells like podocytes and secretory cells (Subramanyam and Krishnamoorthy 1981) have also shown significant changes in their morphology following salinity adaptation. The mitochondrial organization of the gill epithelium also showed distinct changes on adaptation to higher salinity (figure 1). Salinity-adapted crabs basically lost the brush border organization of the gill epithelium. This itself indicates that there is no active salt absorption in the salinity-adapted crabs.

The biochemical data presented in tables 2 and 3 offer evidence to show that the changes occur in the permeability properties of the gill epithelial membranes. $\text{Na}^+ + \text{K}^+$ ATPase activity is known to increase in the gill microsomes of the blue crab, *Callinectes sapidus* following transfer from high salinity to 5‰ salinity (Towle *et al* 1976). In crabs of low salinity, haemolymph NH_4^+ level increases temporarily because of the active excretion of the ion. Besides these, several correlations to show that $\text{Na}^+ + \text{K}^+$ ATPase activity involves uptake of Na^+ across the membrane in return of NH_4^+ into

the medium are given (Mangum *et al* 1976; Weiland and Mangum 1975). The excretory metabolism is therefore related to the ionic fluxes at the gill membrane. When there is Na^+ uptake by the gills in freshwater, obviously the crab consumes more oxygen. It is known that this crab retains a fairly large amount of aspartic acid and glutamic acid in haemolymph, each of which on receipt of one mole of NH_3 , gets converted to neutral asparagine and glutamine enzymatically (Krishnamoorthy and Srihari 1973).

Divalent-ion stimulated ATPase activities were widely used as markers of the functional organization of endoplasmic reticulum (Owens *et al* 1973). Changes in Ca^{2+} and Mg^{2+} ATPase activities in table 4 suggest that there is an impairment of the macromolecular organization of the endoplasmic reticulum in the gills of the crab, as a function of salinity change. Divalent ion stimulated ATPases are further implicated with the composition of membrane lipids, especially phospholipids (Martoonsi *et al* 1968; Owens *et al* 1973). Generally the metal ion ATPases are activated by membrane phospholipids (Meissner and Fleischer 1972) and the cholesterol level influences the ion transport activities of the membrane (De Gier *et al* 1968). The cholesterol/phospholipid molar ratio of the particulate system of the cell or the membranes is an index of membrane permeability. The changes in brush border foldings of apical basal membranes of the lamellar lining may perhaps be interconnected with the enzymic and lipid markers for membrane permeability following salinity adaptations.

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Patterns of fungal resource utilization and feeding range in some mycophagous Tubulifera (Insecta: Thysanoptera)

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Abstract. Adaptive specialization in terms of survival due to diverse fungal feeding preferences and seasonal dispersal is indicated in the sporophagous species *Loyolaia indica* (Anan). Feeding behavioural patterns of six species of mycophagous Tubulifera reflect diversity in fungal resource utilization and feeding range. Influence of fungal food on the strategies of survival, reproduction and aggregation has been discussed.

Keywords. Mycophagous thrips; fungal resource utilization; feeding range; mycophagous tubulifera; *Loyolaia indica*.

1. Introduction

Feeding processes in mycophagous thrips as in phytophagous species involve ecological manipulations, an assessment of which appears to be an important aspect of their biology, particularly in view of feeding and growth being active, dynamic processes. Some sporophagous species like *Loyolaia indica* (Anan.) respond to changing environments by altering their physiological and behavioural processes entering reproductive diapause followed by efficient dispersal to more congenial niches with more adequate fungal food resources. The patterns involved in the feeding process particularly concerning the range of mycophagy — narrow *versus* wide, and associated exploitation of niches both for survival and reproduction also appear important. That reproductive strategies form an integral part of the adaptive strategy of individuals of a particular species has been confirmed in terms of their ability to reproduce by oviparity, ovoviviparity and viviparity (Ananthakrishnan *et al* 1983a). While considerable information is available regarding phytophagous thrips, that relating to feeding preferences and their influence on physiological and behavioural processes in mycophagous thrips appears very meagre (Joshi 1974; Ananthakrishnan *et al* 1983b and Ananthakrishnan and William James 1983). An attempt has, therefore, been made to assess aspects relating to fungal resource utilization in terms of their availability in specific niches as well as the patterns involved in such utilization.

2. Materials and methods

Adults, larvae, pupae and unhatched eggs of *Loyolaia indica* (Anan) *Tiarothrips subramanii* (Ramk.), *Dinothrips sumatrensis* Bagnall, *Priesneriana kabandha* (Ramk.), *Elaphrothrips denticollis* (Bagnall) and *Hoplothrips fungosus* Moulton, were collected from the field, reared in the laboratory with their natural fungus infected host material as food. The stock materials were maintained in BOD incubator at 27°C. For identification of specific species of fungi associated with the mycophagous thrips, the host scrapings and hand sections of the fungus infected host

materials (for identification of fructifications embedded inside the host materials) were stained with lactophenol blue, mounted in lactophenol and identified. Host washings, washed host materials and the gut washings were subsequently plated on potato dextrose agar and oat meal agar media.

The specific feeding sites of the mycophagous thrips in the host material were fixed by observing the location of the colony/individuals, and cut pieces transferred to a 100 ml Erhlenmeyer flasks containing sterile distilled water (25 ml) over an UV lamp. This was shaken vigorously so as to bring the surface mycoflora into the sterile distilled water resulting in host washings. Once sterile distilled water washed host bits were transferred to another 100 ml Erhlenmeyer flask having sterile distilled water, washed well so as to remove any more surface fungi. This procedure was repeated by transferring the bits to 6-7 flasks containing sterile distilled water and washing them successively. The gut of the different sporophagous thrips was dissected out and washed with sterile distilled water. The dissected gut was slit open and the contents were released under aseptic conditions, diluted in sterile distilled water and plated on potato dextrose agar and oat meal agar media. The emerging individual colonies in the petri plates were picked out and transferred to the petri plates containing fresh media and individual pure cultures were obtained for identification. Comparisons were made of the fungi in the host sections, host scrapings, plating of washed host material and plating of host washings to gut spore content and the fed fungal species were identified.

3. Observations

Adaptive specialization in terms of thrips inhabiting specific niches on fungus infected hosts with preference to drying culms of grasses during acute summer, with a tendency to migrate to other hosts with the onset of more favourable conditions when diverse fungi become available, is typical of the species *Loyolaia indica*. Their restriction to drying grass tussocks as well as fungal infected basal drying sheaths of different grasses is correlated with a reproductive diapause when only adults are available during June-September. With the onset of more favourable conditions in September (28-33°C, 72% RH), the individuals reproduce oviparously, each adult female laying 2-5 eggs on hosts such as *Cynodon dactylon* Pers., *Panicum repens* L. and *Chloris barbata* SW. During the reproductive diapause (June-September) the adults survive in drying grass clumps consuming spores of *Fusarium oxysporum* and *Penicillium* sp., while during favourable seasons they feed on the spores of *Lojkania cynodontifolii* (on *Cynodon dactylon*), *Haplosporella* sp. (on *Chloris barbata*), as well as on bicelled spores and multicelled spores (on *Panicum repens*), which appear essential for reproductive fitness. An analysis of the mycoflora at the niches occupied and the specific fungal spores consumed during favourable and unfavourable conditions revealed the relative preference to specific fungal species among the spectrum of fungal species available as indicated in table 1. Dispersal from drying grass clumps in the fields to the drying basal leaf sheaths of *Cynodon dactylon* (October-June) and *Panicum repens* (November-March) in the bunds appears characteristic of this species. In April-May the species occurred dispersed in the drying basal leaf sheaths of *Chloris barbata* (figure 1). Studies on the life cycle of this species on the grass hosts showed the mean duration to be around 15 days (incubation period of the eggs 3-4 days; 1st larvae 2-3 days; 2nd larvae 4-5 days; prepupa 1 day; 1st pupa 1-2 days; 2nd pupa 2-3 days). The change of microhabitats during different seasons, completion of life cycles during a particular season and the diverse feeding range of this thrips appear to be adaptive strategies.

Table 1. Comparative analysis of the fungal species fed by some species of mycophagous Thysanoptera.

Thrips sp.	Host	Fungal spores present	
		at the feeding site	in the gut (qualitative) (+ / -)
<i>Tiarothrips subramanii</i>	Dry leaves of <i>Borassus flabellifer</i>	<i>Anthostomella phoenicicola</i>	+
		<i>Anthostomella sepelibilis</i>	+
		<i>Anthostomella consanquinea</i>	+
		<i>Pestalotia algeriensis</i>	+
		<i>Melenographium citri</i>	+
		<i>Diplodia</i> sp.	+
		<i>Alternaria</i> sp.	+
		<i>Gnomonia</i> sp.	+
		<i>Camarosporium</i> sp.	+
		<i>Phoma</i> sp.	+
		<i>Coniothyrium palmarium</i>	-
		<i>Stigmima palmivora</i>	+
<i>Elaphrothrips denticollis</i>	Dry leaves of <i>Tectona grandis</i>	<i>Pestalotia</i> sp.	+
		<i>Phomopsis tectonae</i>	+
	Dry leaves of <i>Areca catechu</i>	<i>Pestalotia</i> sp.	+
		<i>Pythomyces</i> sp.	-
		a pycnidial fungus	-
		<i>Penicillium</i> sp.	-
		<i>Aspergillus niger</i>	-
		<i>Aspergillus</i> sp.	-
<i>Loyolaia indica</i>	Dry twigs of <i>Zizypus oenoplea</i>	<i>Pestalotia</i> sp.	+
	Dry leaves of <i>Cynodon dactylon</i>	<i>Lojkania cynodontifolii</i>	+
	Dry twigs and clumps of grass	<i>Fusarium oxysporum</i>	+
		<i>Penicillium</i> sp.	+
		<i>Torula herbarum</i>	-
		<i>Aspergillus niger</i>	-
		<i>Aspergillus flavus</i>	-
		<i>Aspergillus fumigatus</i>	-
		<i>Mucor</i> sp.	-
<i>Dinothrips sumatrensis</i>	Dry bark of <i>Piper nigrum</i>	<i>Botryodiplodia theobromae</i>	+
		<i>Verticicladium</i> sp.	-
		<i>Aspergillus niger</i>	-
		<i>Penicillium</i> sp.	-
		<i>Mucor</i> sp.	-
	Dry bark of <i>Hevea brasiliensis</i>	<i>Botryodiplodia theobromae</i>	+
		<i>Fusarium oxysporum</i>	-
		<i>Penicillium</i> sp.	-
		<i>Aspergillus niger</i>	-
	Dry bark of <i>Anacardium occidentale</i>	<i>Aspergillus niger</i>	-
		<i>Botryodiplodia</i> sp.	+
		<i>Fusarium oxysporum</i>	-
		a pycnidial fungus	-

Table 1 (Contd.)

	Dry bark of <i>Mangifera indica</i>	<i>Botryodiplodia theobromae</i>	+
		<i>Candida</i> sp.	-
		<i>Mucor</i> sp.	-
		Lichen	-
<i>Lannea coramandelica</i>		<i>Botryodiplodia theobromae</i>	+
		<i>Fusarium oxysporum</i>	-
		<i>Penicillium</i> sp.	-
<i>Mallotus alba</i>		<i>Botryodiplodia theobromae</i>	+
		<i>Trichothecium roseum</i>	-
		<i>Graphium</i> sp.	-
		<i>Verticilladium</i> sp.	-
		<i>Penicillium</i> sp.	-
<i>Priesneriana kabandha</i>	Dried thorny twigs	<i>Rhytidhysterium rufula</i>	+
	Dead bark of <i>Eucalyptus globulus</i>	<i>Cytospora</i> sp.	+

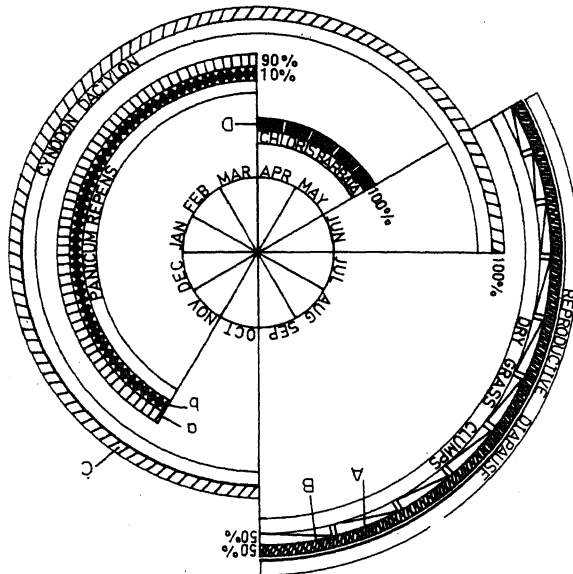


Figure 1. Seasonal incidence of *Loyolajia indica* on different grass hosts and gut spore composition when fed on different hosts A. *Fusarium oxysporum* B. *Penicillium* sp. C. *Lojkania cynodontifolii* a. bicelled spores b. 2-celled spores D. *Haplosporella* sp.

For a better understanding of niche diversity in relation to fungal feeding preference, an assessment of the feeding preference of four species of sporophagous thrips is made. An extreme case of specialization in food preference is offered by *Dinothrips sumatrensis* which when observed on the drying barks of different hosts (such as *Hevea brasiliensis* M. Arg., *Mallotus albus* M. Arg., *Lannea coramandelica* (Houtt.) Merril., *Piper nigrum* L., *Anacardium occidentale* L., and *Mangifera indica* L.) appear to feed only on *Botryodiplodia theobromae*. Niche diversity becomes a necessity to such species which have a highly specialized food preference. An opposite picture is evident in *Tiarothrips subramanii* inhabiting a single host (*Borassus flabellifer* L.) but showing a wider spectrum of fungal food utilization. Gut spore analysis of individuals of

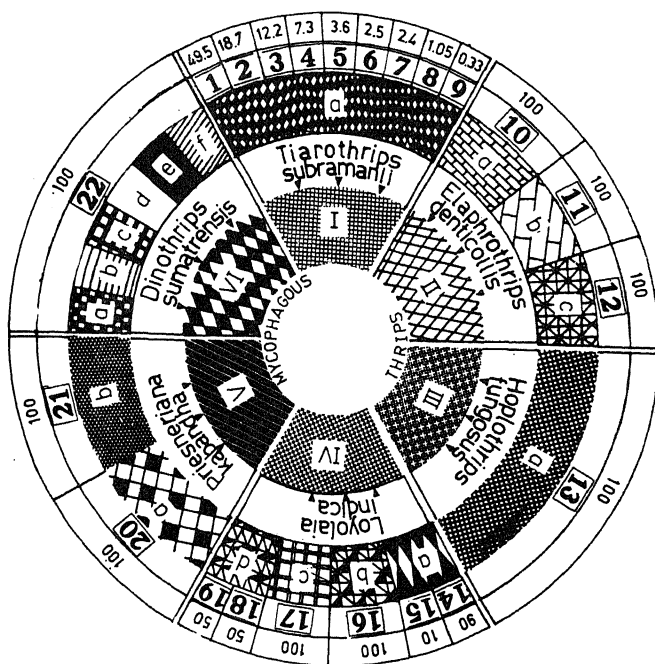


Figure 2. Thrips species, habitat diversity, fungal diversity and percentage of gut spore composition. I. *Tiarothrips subramanii* a. *Borassus flabellifer* 1. *Anthostomella consanguinea* 2. *Phoma* sp. 3. *Diplodia* sp. 4. *Anthostomella phoenicicola* 5. *Stigmina palmivora* 6. *Anthostomella sepeliblis* 7. *Pestalotia algeriensis* 8. *Gnomonia* sp. 9. *Melanographium citri* II. *Elaphrothrips denticollis* a. *Tectona grandis* 10. *Phomopsis tectonae* b. *Zizypus oenoplea* 11. *Pestalotia* sp. c. *Areca catechu* 12. *Pestalotia* sp. III. *Hoplothrips fungosus* a. *Polyporus* sp 13. *Polyporus* sp. IV. *Loyolaia indica* a. *Panicum repens* 14. bicelled spores 15. 4-celled spores b. *Chloris barbata* 16. *Haplosporella* sp. c. *Cynodon dactylon* 17. *Lojkania cynodontifolii* d. Dry grass clumps 18. *Fusarium oxysporum* 19. *Penicillium* sp. V. *Priesneriana kabandha* a. *Eucalyptus globulus* 20. *Cytospora* sp. b. Dry thorny twigs 21. *Rhytidhysterium rufula* VI. *Dinothrips sumatrensis* a. *Hevea braziliensis* b. *Piper nigrum* c. *Lannea coromandelica* d. *Mallotus albus* e. *Mangifera indica* f. *Anacardium occidentale* 22. *Botrydiplodia theobromae* (Numbers in the outermost circle indicate percentage of spores in the gut).

natural population as well as the experimental feeding of the individuals on isolated fungi revealed the relative preferences among fungal species consumed (figure 2). Differences in the feeding preferences of individuals inhabiting different microhabitats of the same host (such as leaf folds, partially drying leaves, fallen and dried leaves of *Borassus flabellifer*) which harbour different species of fungi are equally evident; their presence in the folds of drying leaves throughout the year appears to provide an ideal microhabitat for the survival of the feeding thrips species.

Equally interesting are the species *Elaphrothrips denticollis* (which so far has been observed appears to feed on only *Pestalotia* sp. and *Phomopsis tectonae*) and *Priesneriana kabandha* (known to feed on *Rhytidhysterium rufula* and *Cytospora* sp.) which have restricted food and host preferences as indicated in figure 2. Mycetophagous *Hoplothrips fungosus* known to occur in a wide range of habitats, also appear to utilize the fructifications of *Polyporus* sp., the females laying their eggs within the numerous comb like structures of the fungus thereby ensuring sufficient food for the developing larvae. Other *Hoplothrips* species known to inhabit woody

shelf fungi (*Polyporus* sp., *Ganoderma* sp. and *Sterem* sp.) are *Hoplothrips americanus* (Hood), *Hoplothrips angusticeps* (Hood) *Hoplothrips beachae* (Hinds) and *Hoplothrips terminalis* (Hood and Williams) (Graves and Graves 1970).

3.1 Feeding behaviour and aggregation pattern

Observations on the different thrips studied showed differences in the aggregation/dispersal pattern correlated with the food range diversity. *Tiarothrips subramanii*, *Elaphrothrips denticollis* and *Priesneriana kabandha* revealed aggregation/dispersal behaviour depending on the microhabitats occupied. Linear aggregations (3-1 cm $\times$ 0.5-1 cm) were typical of *Tiarothrips subramanii* within the folds of *Borassus flabellifer*, the dispersal pattern being evident on open surfaces of the leaves. Similarly *Priesneriana kabandha* and *Elaphrothrips denticollis* which form aggregations in the subcortical layers of *Eucalyptus globulus* and folded leaves of *Areca catechu* respectively, showed a dispersed pattern when on the surfaces of bark and leaves. The fungal flora in the diverse niches occupied by these thrips, as well as the nature of the consumed spores seem very different. In such microhabitats where the conditions appear conducive to luxuriant fungal growth as well as to the thrips (leaf folds and subcortical areas), aggregation behaviour by these thrips evidently appears typical. Dispersed patterns appear to be more characteristic with inhabiting leaf surfaces when conditions become more favourable for the growth of different fungi. In *Dinothrips sumatrensis*, though pupae were seen to aggregate, the larvae and adults tend to be dispersed, correlating with the restricted feeding range and openness of the microhabitats occupied and diversity in the fungal habitats. *Loyolaia indica*, a non-aggregating species, has a diversity in feeding range and host range as indicated earlier, and the influence of diverse fungal food consumed on their life cycle and reproductive fitness appears considerable. Observations on the feeding sites, fungal resources utilization, feeding range and dispersal by *Loyolaia indica* on different hosts during different seasons, revealed that dispersal is facultative being triggered by abiotic factors. Movements by species like *Loyolaia indica*, *Priesneriana kabandha* and *Elaphrothrips denticollis* could be categorised under dispersive movements, since they lack the behavioural patterns typical of migrants. In other words, it is a movement that occurs when the requirements of thrips change due to differences in feeding and microclimate (Southwood 1962).

4. Discussion

In phytophagous insects, the favoured host plant is something 'lived on' with the whole community as such being selected, so that the interaction between the host and the insect is direct and preference by the insect being towards survival, food and the community as a whole (Dethier 1954). In mycophagous thrips, the situation is further complicated in that the interaction is triangular, involving the insect species, the preferred microhabitat where the insect 'lives' and the fungal species that is fed upon. Hence, the preferred microhabitat by harbouring the needed specific fungal species as well as by providing the needed ideal microclimate, may directly/indirectly influence the survival of the thrips. Observations on feeding range and host preferences of six species of mycophagous thrips indicated diversity of patterns so that the trends in feeding and host preferences assume distinct advantageous adaptations for the survival of these thrips. A major observation that emerged from the analysis of food

range and host preferences relates to the various categories of food range diversity among mycophagous thrips studied here *i.e.*, (a) highly restricted food range and niche diversity (*e.g.*, *Dinothrips sumatrensis*), (b) restricted food range and niche variability (*e.g.*, *Priesneriana kabandha* and *Elaphrothrips denticollis*) (c) diversity in food range and restricted niche variability (*Tiarothrips subramanii*) and (d) diversity in food range and niche variability (*e.g.*, *Loyolaia indica*). The observations also revealed that restricted food range and/or relative preference of food in different niches is generally coupled with niche-diversity, while diversity in food range is coupled with restricted niche variability. The restriction or relative preferences in fungal food may necessitate mycophagous thrips dispersing to various hosts which might harbour the preferred fungal species, while for the thrips having diverse food range and occupying an ideal microhabitat (which harbours the needed fungal species), dispersal to different sites appear unwarranted. Further, the nature of the microhabitat coupled with the tolerance/resistance of the host to the growth of specific species of fungi might exert a tremendous influence on the host preferences by mycophagous thrips. Nutritional factors as the basis for restriction/diversity in feeding preferences may be explained in thrips species showing a narrow or wide feeding range. The preference of spores to mycelia, the restricted preferences towards higher fungi and the preferential feeding of Coelomycetes and Hyphomycetes by some sporophagous thrips (having restricted feeding range) may have to be ultimately explained on the basis of nutrition, a factor which has to involve considerable amount of experimental evidence.

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Ammonia and urea excretion in three species of air-breathing fish subjected to aerial exposure

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Abstract. Proportions and rates of excretion of ammonia and urea and their levels in blood and liver were determined in two obligate (*Anabas scandens* and *Channa gachua*) and one facultative (*Mystus vittatus*) air-breathing teleosts. *A. scandens* and *C. gachua*, when exposed to air and returned to water, showed marked increase in the proportions of excretion of ammonia N and urea N, the increase in the rate of excretion of urea N being much greater. Significantly high urea N levels in blood and liver were noticed in both species following emersion. Extra synthesis of urea and marked shift towards ureotelism seem probable in exposed *A. scandens* and *C. gachua*. No such adaptive shift was observed in *M. vittatus*. Ability to survive for longer periods out of water by *A. scandens* and *C. gachua* may be attributed to this adaptive shift besides other physiological adaptations.

Keywords. *Anabas scandens*; *Channa gachua*; *Mystus vittatus*; ammoniotelism; ureotelism.

1. Introduction

Nitrogen metabolism is considered one of the most sensitive physiological systems showing adaptive responses to environmental variations. A major feature of these responses is reflected in the change in relative amounts of the most important nitrogenous excretory products with changes in the availability of water supply (Gordon 1968).

Air-breathing teleosts suffer from elimination of gaseous ammonia during periods out of water, warranting a mechanism of detoxification of ammonia. A shift from ammoniotelism to ureotelism was reported in air-breathing teleosts, *Periophthalmus sobrinus*, *P. cantonensis* (Gordon *et al* 1969, 1978) and *Sicyases sanguineus* (Gordon *et al* 1970) upon emersion. The present paper examines whether such a shift takes place in the air-breathing teleosts *Anabas scandens*, *Channa gachua* and *Mystus vittatus* under experimental conditions.

2. Materials and methods

Samples of *A. scandens* (16-30g), *C. gachua* (16-35g) and *M. vittatus* (9-16g) collected from local ponds were maintained in separate cement cisterns at $28 \pm 1^\circ\text{C}$ and fed with boiled eggs and earthworms alternatively, every two days. Feeding was discontinued one day before the experiment. To study the proportions and rates of excretion of ammonia and urea in normal fishes, each fish was introduced into a glass jar

containing 150 ml water and the water samples collected (in triplicate) after 5 hr were analyzed for ammonia and urea. Effect of exposure on ammonia and urea excretion was studied by introducing two groups of fish exposed to air (80% relative humidity) for 5 and 10 hr (only 5 hr in the case of *M. vittatus*), each into a glass jar containing 150 ml water and analyzing the water for ammonia and urea after 5 hrs. Ammonia in the water was estimated colorimetrically by direct Nesslerization (Hawk *et al* 1954). Urea content was determined by treating the water with urease and estimating the ammonia formed by direct Nesslerization (Hawk *et al* 1954). The data obtained are expressed in mg N/kg/hr.

Deproteinized tungstic acid filtrate of the blood was used to analyse the ammonia and urea content of blood. Ammonia was estimated by the microdistillation method of Folin and Swedberg (1930). Urea in the filtrate was treated with urease and the ammonia released was estimated as detailed earlier.

To estimate the ammonia and urea in liver, about 100 mg of liver tissue was homogenized and deproteinized with dilute tungstic acid. The filtrate was analysed by microdistillation method as described earlier. Levels of ammonia and urea in blood were expressed as mg N/100 ml of blood and in liver as mg N/gm wet weight. The data were analyzed for significance using *t* test.

3. Results

A. scandens, while in water, normally excreted larger amounts of ammonia than urea (ammonia N:urea N ratio, 3:2). When returned to water after 5 hr of exposure, ammonia N and urea N increased significantly by about 1.5 and 2 times respectively. The ammonia N:urea N ratio in these fish was 1:1. When exposed to air for 10 hr and returned to water, significant increase in ammonia N (2.5 times) and urea N (4 times) was noticed (ammonia N:urea N, 1:1.3) (table 1). From the ratios of ammonia N:urea N excreted by the fish in water and fish returned to water following exposure (table 1), a gradual shift in the production of nitrogenous waste from ammonia to urea was observed.

Table 1. Rate of excretion of ammonia N and urea N in *Anabas scandens*, *Channa gachua* and *Mystus vittatus*.

Experimental Conditions	Rate of excretion mgN/kg/hr		Ammonia N: Urea N ratio
	Ammonia N	Urea N	
<i>Anabas scandens</i>			
Normal	11.06 (15)	8.37 (15)	3:2
5 hr exposed	16.24*(15)	16.35*(15)	1:1
10 hr exposed	26.95*(11)	33.86*(11)	1:1.3
<i>Channa gachua</i>			
Normal	7.13 (14)	9.91 (14)	1:1.2
5 hr exposed	8.89 (10)	13.26*(10)	1:1.5
10 hr exposed	14.03*(12)	50.14*(12)	1:3.6
<i>Mystus vittatus</i>			
Normal	10.59 (12)	7.78 (12)	1:4.1
5 hr exposed	37.77*(12)	18.29*(12)	2:1

* Significant at 5% level; figures in parentheses indicate the number observations.

Table 2. Concentrations of ammonia N and urea N in blood and liver of *Anabas scandens*, *Channa gachua* and *Mystus vittatus* under normal and experimental conditions.

	Concentration in blood (mgN/100 ml of blood)			Concentration in liver (mgN/ w/w of liver)		
	Amm. N	Urea N	Amm. N:Urea N ratio	Amm. N	Urea N	Amm. N:Urea N ratio
<i>Anabas scandens</i>						
Normal	1.96	3.13	2:3	0.09	0.14	1:1.5
5 hr exposed	3.21*	12.79*	1:4	0.09	0.34*	1:4
10 hr exposed	2.44	14.04*	1:6	0.07	0.50*	1:7
5 hr exposed and 5 hr in water	1.53	1.51	1:1	0.19*	0.18	1:1
10 hr exposed and 5 hr in water	2.97	4.76*	1:1.5	0.04	0.18	1:4.5
<i>Channa gachua</i>						
Normal	1.86	3.89	1:2	0.08	0.07	1:1
5 hr exposed	3.04*	3.55	1:1.2	0.09	0.08	1:1
10 hr exposed	1.32*	9.98*	1:7.5	0.04*	0.24*	1:6
5 hr exposed and 5 hr in water	1.69	4.09	1:2.4	0.08	0.07	1:1
10 hr exposed and 5 hr in water	2.17	3.63	1:1.7	0.06	0.09	1:1.5
<i>Mystus vittatus</i>						
Normal	0.19	0.17	1:0.9	0.07	0.04	2:1
5 hr exposed	0.35*	0.32*	1:1	0.06	0.05	3:4
5 hr exposed and 5 hr in water	0.16	0.16	1:1	0.06	0.05	1:1

* Significant; Each value represents the mean of 12 observations.

Blood concentrations of ammonia and urea in fish in water showed a higher level of urea N (ammonia N:urea N, 2:3). Exposure of fish for 5 hr caused only 1.5 fold increase in ammonia N while urea N increased 4 times (ammonia N:urea N, 1:4). When exposed for 10 hr, urea N level showed further increase to 4.5 times with a ratio of 1:6 (table 2).

The ratio of ammonia N:urea N in the liver of normal fish was 1:1.5. While no significant accumulation of ammonia N was observed in the liver of fish exposed for 5 hr, urea N increased by about 2.5 times (ratio, 1:4). When exposed for 10 hr, ammonia content of liver showed no significant change whereas urea N increased by about 3.5 times (ratio, 1:7). When exposed for 5 hr and returned to water and maintained for 5 hr urea N dropped to normal levels whereas ammonia N showed significantly higher levels. On the contrary, those fish exposed for 10 hr and returned to water for 5 hr showed normal levels of ammonia N and higher levels of urea N (table 2).

C. gachua, when maintained in water, excreted more urea N. Exposure of fish for 5 hr resulted in no significant change in the rate of ammonia N excretion upon return to water whereas urea N excretion increased significantly to about 1.5 times (ratio, 1:1.5). Exposure for 10 hr and subsequent return to water resulted in 2 to 5 fold increase in the excretion rates of ammonia N and urea N respectively (ratio, 1:3.6).

Concentrations of urea N were greater than that of ammonia N in the blood of normal fishes (ratio, 1:2). When exposed for 5 hr, ammonia level increased to about 1.6 times. The significant accumulation of ammonia N in fish exposed for 5 hr together

with the lack of corresponding increase in the rate of ammonia N in fish exposed for 5 hr probably indicates the conversion of accumulated ammonia N to less toxic substances such as urea. Fish exposed for 10 hr showed significant decrease in ammonia N and significant increase in urea N (ratio, 1:7.5). Normal levels of ammonia N and urea N were restored in blood when fish exposed for 5 and 10 hr were returned to water and maintained for 5 hr.

The ratio of ammonia N:urea N in the liver of fish in water was 1:1. Exposure for 5 hr resulted in no change in the concentrations in the liver while 10 hr exposure resulted in 50% reduction in ammonia N and 3-fold increase in urea N. Probably this indicates conversion and storage of urea N under exposure stress. When returned to water and maintained for 5 hr these levels restored normalcy.

M. vittatus normally excreted more ammonia N than urea N (ratio, 1.4:1). When exposed for 5 hr the rate of ammonia N excretion (3.6 times) and urea N (2.4 times) increased.

Increase in ammonia N and urea N levels (1.8 times) in the blood resulted in 5 hr exposure. In the liver, only urea N showed increase (2-fold) consequent to exposure.

4. Discussion

If it is assumed (Gordon *et al* 1969) that during exposure of the fish, the nitrogen metabolism does not alter and the nitrogenous wastes are not excreted during exposure, the rates of excretion of ammonia N and urea N should get doubled and trebled on return to water for 5 hr following 5 and 10 hr of exposure respectively. Similarly the levels of ammonia N as well as urea N in the blood and liver of the fish exposed for 5 hr should get doubled and for those exposed for 10 hr should get trebled. Lesser values mean a drop in the production of these wastes and higher values would mean extra synthesis.

A perusal of tables 1 and 2, based on the above assumption, indicates that there is expected increase in the urea N excretion following 5 hr (2-fold) and 10 hr (4-fold) of exposure of *A. scandens*. The increase in urea N in blood is also more than doubled and trebled in *A. scandens* exposed for 5 and 10 hr respectively (table 2). In *C. gachua*, however, a similar expected increase of urea N in liver and blood and subsequent increased rate of excretion of urea N are observed only upon exposure for 10 hr. Extra synthesis of urea and subsequent increase in the rate of excretion of urea N during prolonged exposure probably suggest ureogenesis and tendency towards ureotelism in *A. scandens* and *C. gachua*. The absence of proportionate increase of ammonia in blood and liver also suggest the conversion of ammonia to urea.

Ammonia continued to be the predominant nitrogenous waste in *M. vittatus* even after 5 hr of exposure unlike in the other two species. The levels of urea N and ammonia N accumulated in tissues following exposure were not significantly high indicating no extra synthesis. It is probable that a synthetic pathway for urea production and an adaptive shift towards ureotelism are lacking in this species.

A. scandens and *C. gachua* are obligate air-breathers living in stagnant and restricted bodies of water and are capable of surviving longer periods out of water (*A. scandens* for 22 to 25 hr (Reddy and Natarajan 1971) and *C. gachua* for 18 hrs (Ramaswamy and Reddy 1978). These species share many features with reference to nitrogen excretion. The shift towards ureotelism compare with that of the amphibious fish, *Periophthalmus sobrinus* (Gordon *et al* 1969), *Sicyases sanguineus* (Gordon *et al* 1970), *Periophthalmus cantonensis* (Gordon *et al* 1978), the dipnoan, *Protopterus*

aethiopicus (Smith 1930) and the aquatic anurans, *Xenopus laevis* and *Bufo calamita* (Balinsky *et al* 1961, 1967), although the degree varies. Ecologically such shifts are advantageous to these fish to overcome the drought conditions of the tropical ponds where they live.

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Seasonal variation in the dimension of scent-marking gland of three desert rodents and its possible relationship with their reproductive performance

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Abstract. The two gerbils, *Tatera indica* and *Meriones hurrianae*, and the metad, *Rattus meltda pallidior* exhibit significant monthly variations in the dimension (maximum length $\times$ maximum breadth) of the ventral scent marking gland. Peaks in gland size occur late in winter and monsoon which generally correspond to the maximum breeding activity of these rodents during the year. However, the monthly averages of gland area and incidence of pregnancy in the three species do not follow the same trend. It is difficult, therefore, to conclude if the scent-marking behaviour and the breeding performance of the rodents are inter-related.

Keywords. Scent marking gland; breeding season; olfactory communication; *Tatera indica*; *Meriones hurrianae*; *Rattus meltda*

1. Introduction

Scent-marking by rodents through cutaneous glands has been attributed to a number of functions pertaining to olfactory communication among them. Whether odour of the exudation of ventral scent marking gland plays an important role in the reproductive activity of mammals is not clear. However, it has been found that the frequency of scent-marking in the female desert gerbil, *Meriones hurrianae* enhances significantly ($P < 0.01$) during pro-oestrus and oestrus as compared to that when the females are in dioestrus (Kumari and Prakash 1981) and scent-marking appears to have a function of advertising the ready-to-mate state of females. Similar observations have been recorded in the female Indian gerbil, *Tatera indica* which possess the mid-ventral marking gland (Prakash and Idris 1982). In certain other mammals also, it is reported that the gland activity and marking behaviour increase considerably during breeding season (Eibl-Eibesfeldt 1953; Erlinge 1968; Mykytowycz 1966; Stoddart 1972; Townsend 1953). Contrary to this, evidence is available that scent glands are active throughout the year, irrespective of breeding season among other mammals (Pearson 1946; Sokolov and Skurat 1966). An attempt to examine the relationship between the gland size and reproductive activity of three field rodents inhabiting the desert region was made and the results are presented in this paper.

2. Methods

Field rodents, *T. i. indica*, *M. hurrianae* (Gerbillinae) and the soft-furred field-rat or the metad, *Rattus meltda pallidior* (Murinae) were collected from desert grasslands during 1979, 1980 and 1981. The body weight of the freshly captured animals, their

gland size (maximum length $\times$ maximum breadth) and reproductive status were recorded for every freshly-captured rodent. The testes weight were determined on a semi-micro balance with an accuracy of 0.001 g. Statistical methods followed for determining correlation coefficient and analysis of variance are after Snedecor and Cochran (1967).

3. Results

The ventral scent-marking gland is present in both the sexes of *M. hurrianae*, in the males of *T. indica* and *R. m. pallidior*. It also occurs in a few females of *T. indica*.

The gland area of male *T. indica* between months fluctuate significantly as evidenced by the analysis of variance ($F_{11,38} = 11.48$, $P < 0.01$). The mean monthly gland area exhibits three peaks, during February, April and September, and is the lowest during November and December (figure 1). When the data are pooled on a seasonal basis, it is

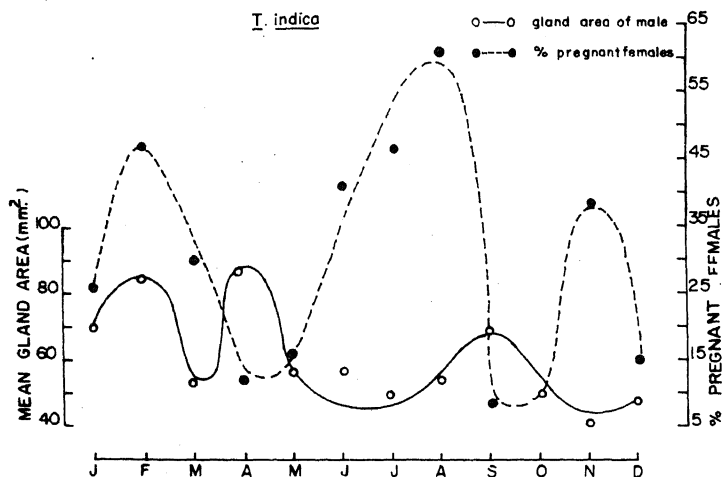


Figure 1. Monthly fluctuations in the dimensions of scent marking gland of male *T. indica* and percent incidence of pregnancy.

Table 1. Seasonal variations in the area of scent marking gland and incidence of pregnancy in *T. indica* and *R. m. pallidior*.

Season	<i>T. indica</i>		<i>R. m. pallidior</i>	
	Average gland area of male (mm ²)	% pregnant females	Average gland area of male (mm ²)	% pregnant females
Winter (Dec-Feb)	67.9	29.3	157.3	59.7
Summer (March-June)	64.0	24.7	195.2	61.3
Monsoon (July-Sept)	58.3	38.6	160.6	61.1
Post-monsoon (Oct-Nov)	46.4	24.0	190.0	63.6

evidenced that the gland area in male rodents is maximum during winter and gradually declines till post-monsoon (table 1).

A comparison of mean monthly gland area of male *T. indica* and the incidence of pregnancy (Jain 1970) indicates quite an inverse relationship between the two. When the gland size is largest in April (figure 1), the pregnancy rate is fairly low and when the latter is maximum during June-August period, the gland area of male *T. indica* shows a downward trend. Similarly, when the gland area is minimum during November the frequency of pregnant females is high (38%). Similarly in September, when the gland area shows a peak, the incidence of pregnancy is lowest (9%). Surprisingly the seasonal data indicate a slight similarity in the fluctuation trend between the two (table 1).

Mean monthly gland area of the desert gerbil, *M. hurrianae* ranges in males from 80.8 to 160 mm² and among females from 35 to 104 mm². Analysis of variance indicates that the differences between months ($F_{11,149} = 3.75$, $P < 0.05$), between sexes

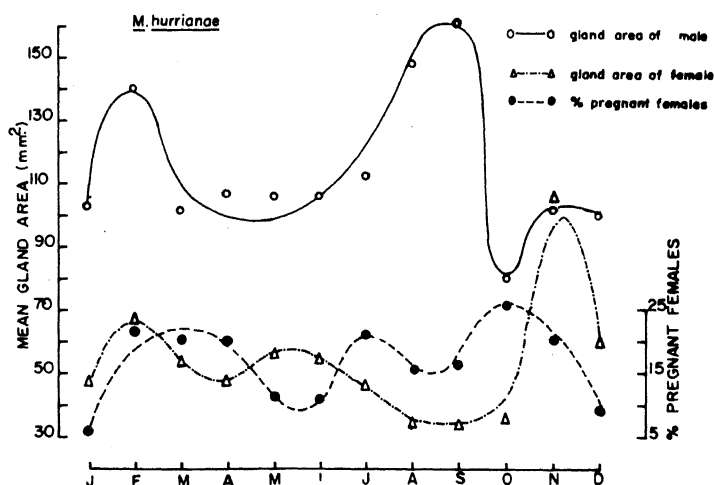


Figure 2. Monthly fluctuations in the dimensions of scent marking gland of male and female *M. hurrianae* and percent incidence of pregnancy.

Table 2. Seasonal variations in the area of scent marking gland and incidence of pregnancy in *M. hurrianae*.

Season	<i>M. hurrianae</i>		% pregnant females
	Average gland area (mm ²) male	female	
Winter (Dec-Feb)	118.1	59.3	16.8
Summer (March-June)	105.4	54.7	16.0
Monsoon (July-Sept)	140.2	38.9	18.6
Post-monsoon (Oct-Nov)	91.4	70.4	23.3

($F_{1,149} = 105.93$, $P < 0.001$) and interactions between month and sexes ($F_{11,149} = 6.29$, $P < 0.001$) are significant. The gland size of male and female desert gerbil follows almost a similar trend from January to June, both showing a peak in February. Thereafter, the gland size in females declines up to October, showing a peak again in November. However, in male animals, from June onwards, till September the gland size increases steeply, thereafter it falls and corresponds with the maximum breeding epoch of females (figure 2). However, the receding gland area in females during this period is rather perplexing. The analysis of data on season-wise basis reflects this difference more clearly (table 2); whereas the gland area is maximum in male desert gerbils, it is minimum in females during monsoon when their breeding activity is sustained at a higher level (Kaul and Ramaswami 1968).

The mean monthly gland area in male *R. m. pallidior* exhibits peaks in March-April, July and November and is low in May-June, August, and September (figure 3). The variations between the months are significant ($F_{10,82} = 8.992$, $P < 0.01$). The trend of monthly variations in gland area is almost reverse to that of frequency of pregnancy (figure 3). During October the incidence of pregnancy is maximum (Rana and Prakash 1982) but the gland area is fairly low. At 47% pregnancy rate (September) the gland area is low, whereas when the gland area is highest (March, April), the pregnancy rate is 58.7 and 62.2% respectively. In February and May when pregnancy frequency is 66.6

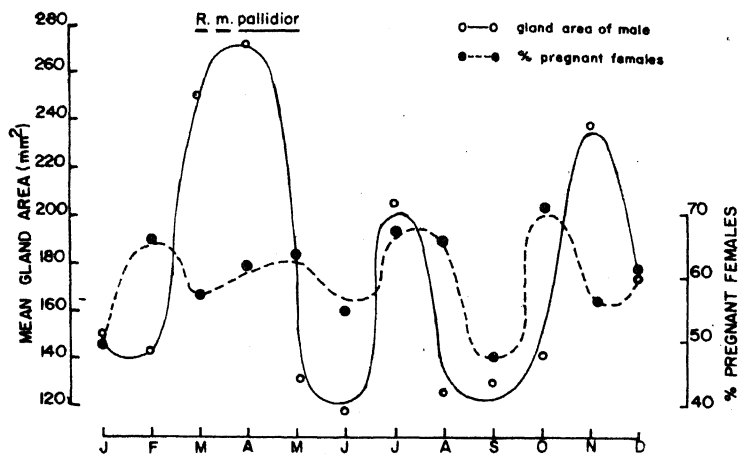


Figure 3: Monthly fluctuations in the dimensions of scent marking gland of male *R. m. pallidior* and percent incidence of pregnancy.

Table 3. Relationship between gland area and testicular weight.

Species	Mean $\pm$ S.E. Gland area (mm ²)	Mean $\pm$ S.E. Paired testes weight (mg)	Correlation coefficient (<i>r</i>)
<i>T. indica</i>	60.33 $\pm$ 4.23	1.59 $\pm$ 0.15	+ 0.3776**
<i>M. hurrianae</i>	138.33 $\pm$ 12.26	0.33 $\pm$ 0.03	+ 0.3313**
<i>R. m. pallidior</i>	200.45 $\pm$ 12.03	0.51 $\pm$ 0.09	+ 0.4250**

** Level of significance $P < 0.01$

the gland area is very low, 142 mm² and 136 mm² (figure 3) respectively. The seasonal averages indicate that the proportion of the pregnant females varies very little all the year round but the gland area shows two clear peaks (table 1), one during summer and the other during post-monsoon. Such apparent variations in gland area of male metads were not expected when the incidence of pregnancy did not show significant fluctuations.

On an overall basis, however, the gland area and paired testes weights are significantly ($P < 0.01$) correlated (table 3).

4. Discussion

The two gerbils and the metad exhibit significant ($P < 0.01$) monthly fluctuation in the gland area. This fluctuation trend is more or less similar in the three species with respect to the observed peaks which occur late during winter and monsoon. These peaks also correspond generally to the breeding activity of these rodents which is maximum during these seasons. The overall correlation would suggest that during the maximum breeding period of the rodents, atleast the male glands are larger indicating the increase in the secretory activity of the scent-marking gland and its enhanced role in olfactory perception. Such generally increased scent-marking activity during the breeding season and comparative quiescence during the non-breeding season has also been reported in the wild rabbit, hare (Mykytowycz 1966), in kangaroo rat (Quay 1953) and Mongolian gerbil (Mitchell 1967).

The above conjecture is not quite supported if the monthly averages of gland area and incidence of pregnancy in the three species studied are compared. During a number of months when the gland area is maximum the incidence of pregnancy is very low and *vice-versa* (figures 1 to 3). The seasonal variations in the two do not quite follow the same trend except that for male *M. hurrianae*. Another quite important evidence brought out is that the fluctuation in the gland area of male and female *M. hurrianae* follows entirely different paths; it is maximum in males during monsoon when it is minimum in females (table 2).

Another perplexing phenomenon observed is that when all the three species follow the same trend as far as the reproductive performance is concerned the fluctuations in the gland area differ between the species. If there was any definite relationship between the gland area and breeding activity, the variations in the former are expected to follow almost a similar trend. In a number of other mammals such as Siberian hamster (Vorontsov and Gurtovoi 1959), black-tailed deer (Muller-Schwarze 1971), kangaroo rat (Quay 1953) Mongolian gerbil (Mitchell 1967), white rat (Stanely and Powell 1941) and several artiodactyles (Von-Schumacher 1936; Moy 1969) the secretory activity of the marking glands is reported to be consistent throughout the year irrespective of the breeding seasons.

The results of the above studies and those of the present one provide rather inconclusive evidence about the relationship between the reproductive performance and the dimensions of the ventral scent marking gland of rodents.

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The authors are thankful to Shri S P Malhotra for facilities and encouragement.

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Utilization of dietary protein, fat and carbohydrate by the paddy field fish (*Macropodus cupanus*: Anabantidae)

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Abstract. Preferential utilization of protein, fat and carbohydrate by different weight classes of *Macropodus cupanus* was studied exposing the fish to algal, animal and combined feed. Consumption and conversion of protein and fat was maximum in the liver fed group. At maximum feeding the small fish converted carbohydrate, fat and protein at an efficiency of 9.45, 26.55 and 18.29% respectively. The efficiency of conversion decreased with increasing body weight. At all feeding schedules carbohydrate was converted more or less efficiently. The fish exposed to minimum animal feed or 100% algal feed utilized almost equal amount of stored protein or fat for maintenance metabolism.

Keywords. Dietary protein; fat; carbohydrate; consumption; conversion; *Macropodus cupanus*.

1. Introduction

This paper is a sequel to our previous studies on the effect of food quality on food utilization (Mathavan and Christopher 1980), swimming activity (Christopher and Mathavan, 1980) and proximate body composition (Christopher and Mathavan 1983) in the anabantid air breathing fish *Macropodus cupanus*. Feeding the fish with liver, algae or different combinations of liver and algae, it has been shown that (i) rates (feeding, absorption and conversion) and efficiencies (absorption and conversion) increased with increasing proportion of liver in the feed combination (Mathavan and Christopher 1980); (ii) enhanced surfacing and associated activities in the liver fed group than in other groups (Christopher and Mathavan 1980) and (iii) the existence of significant correlation between water content/feeding rate and body composition (Christopher and Mathavan 1983). The present paper reports the utilization of protein, carbohydrate and fat by the fish *M. cupanus* fed with different combinations of plant and animal food.

2. Materials and methods

The experimental design followed in the present study has been described elsewhere (Mathavan and Christopher 1980). Briefly, fish were grouped into three weight classes (130 ± 50 ; 295 ± 45 , 1000 ± 200 mg). Five series of feeding experiments were conducted in each weight group. Fish of different weight classes were offered the following percentage combinations of animal (goat liver) and algal (*Spirogyra maxima*) food: 100:0, 75:25, 50:50, 25:75 and 0:100 respectively. Fish were fed on the respective food combination for 2 hr per day for 15 to 21 days. All the experiments were performed at $27 \pm 1^\circ\text{C}$ and 14L:10D photoperiod. After the experimental period the test individuals were sacrificed (Maynard and Loosli 1962) and dried at 90°C .

amples of food and fish were powdered and the organic compounds such as protein, and carbohydrate were estimated.

Fat and protein contents were estimated following the method of Folch *et al* (1957) and Lowry *et al* (1951) using bovine serum albumin as standard respectively. Following the method of Dubois *et al* (1956) total carbohydrate was estimated using thionine reagent.

Rates of feeding and conversion of individual components were calculated relating the quantity of protein, fat or carbohydrate consumed/converted to the initial live weight of the fish.

$$\text{Feeding rate (mg g fish}^{-1} \text{ day}^{-1}) = \frac{\text{Consumption} \times 1000}{\text{Initial live weight of the fish} \times \text{duration}}$$

$$\text{Conversion rate (mg g fish}^{-1} \text{ day}^{-1}) = \frac{\text{Conversion} \times 1000}{\text{Initial live weight of the fish} \times \text{duration}}$$

Gross conversion efficiency (K_1) was calculated using the following formula:

$$K_1 (\%) = P/C \times 100$$

where P = production and C = consumption

Results

Chemical composition of goat liver and *S. maxima*, the food materials offered in the present study are given in table 1. Protein content was 40.5% in liver and 14.6% in *S. maxima*. Fat content averaged 25% in the former while in the latter it was only 6%. In *S. maxima* carbohydrate was the major constituent (50%). Since the individuals in the present series received different combinations of algal and animal diet, the percentage composition of fat, protein, carbohydrate and ash in their feed varied in each group. With the increasing supplementation of algae in the feed, percentage composition of protein and fat decreased whereas that of carbohydrate and ash increased (figure 1). Data on consumption and conversion of protein, fat and carbohydrate in different weight classes of *M. cupanus* receiving algal, animal and combined diet are presented in figure 2. The large weight class fed exclusively on liver consumed about 166 mg protein during the experimental period of 21 days. It decreased to 138, 111 and 81 mg in the group receiving 75%L: 25%A, 50%L: 50%A and 25%L: 75%A. Those fed exclusively on algae consumed only about 20 mg of protein during the experimental

Table 1. Percentage composition (mean $\pm$ SD) of water, carbohydrate, protein, fat and ash in the food materials: liver and *Spirogyra maxima* (values are expressed in dry weight).

	Liver	Algae
Water content (%)	74.4 $\pm$ 2.8	94.6 $\pm$ 1.6
Carbohydrate (%)	20.5 $\pm$ 4.4	50.0 $\pm$ 3.1
Protein (%)	40.6 $\pm$ 3.4	14.6 $\pm$ 2.1
Fat (%)	25.0 $\pm$ 4.0	6.0 $\pm$ 1.2
Ash (%)	14.0 $\pm$ 1.9	29.4 $\pm$ 3.0

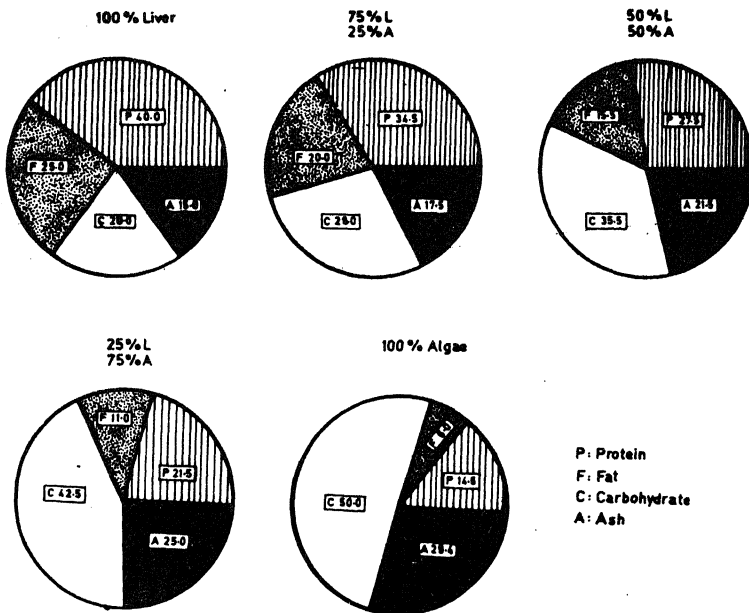


Figure 1. Percentage composition of proteins, fat, carbohydrate and ash in the feed (liver, algae and in combination).

period. Similarly, consumption of fat steadily decreased from 102 mg in the liver fed group to 8 mg in the algal fed group. However, the consumption of carbohydrate did not vary significantly; it was about 84 mg in the liver fed group as against 68 mg in the algal fed group. The pattern of consumption obtained in the small and medium weight group are similar to the one obtained for the large weight group (figure 2; lower panel).

Similar to consumption, conversion of fat and protein decreased with increasing supplementation of algae in the feed in all weight groups (figure 2; upper panel). Critical analysis of the figure reveals that test individuals receiving 100% A and 75% A : 25% L lost considerable amount of reserved protein or fat for maintenance. However, it was more pronounced in the large weight group than in the small weight group (figure 2; upper panel). Smaller or larger amount of carbohydrate was converted at all the feeding levels in the tested weight groups. In fact the large weight class doubled carbohydrate conversion in the series fed exclusively on *S. maxima* (16 mg) as compared to the liver fed series (8 mg).

Feeding rate of carbohydrate was not much altered in the group receiving either liver algae or combined feed (table 2). For instance, the large weight class consumed carbohydrate at the rate of 4.9 mg/g/day or 4 mg/g/day when fed on liver or algae respectively. Rate of protein or fat intake was significantly reduced in algal fed series than in liver fed series. The protein consumption was 21 mg/g/day in the small weight class fed exclusively on liver and it was only 3 mg/g/day for the same group receiving *S. maxima* alone as food (table 2).

Figures 3, 4 and 5 represent the rate of conversion of protein, fat and carbohydrate in *M. cupanus* exposed to different feeding schedules of animal and algal food. The interesting results are: (i) maximum rate of carbohydrate conversion was observed in

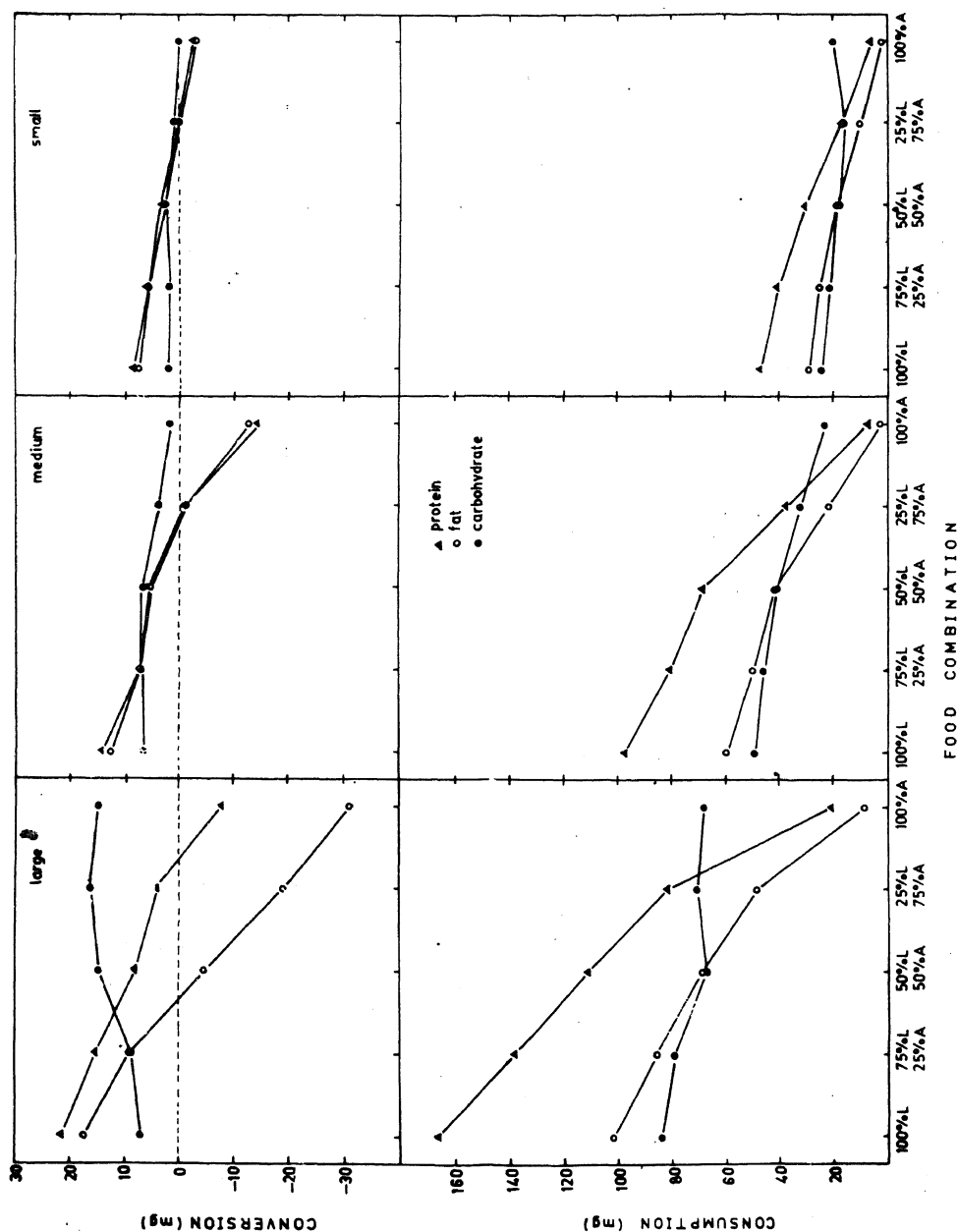


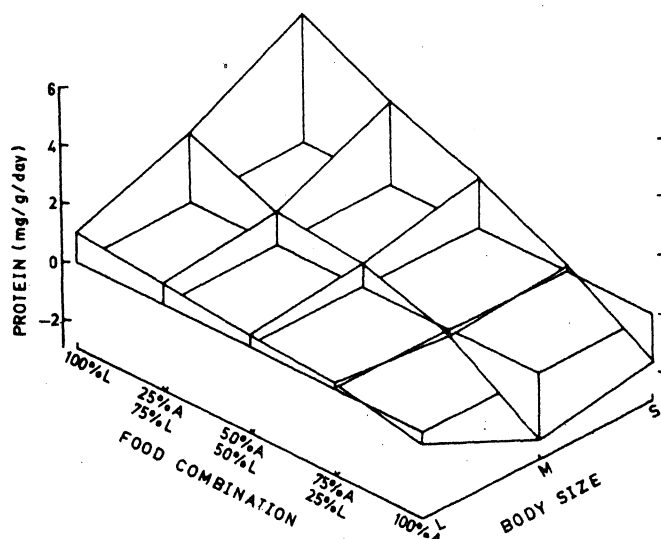
Figure 2. Consumption (lower panel) and conversion (upper panel) of proteins, fat and carbohydrate by large, medium and small weight classes of *M. cupanus* exposed to animal, algal and combined feed for the experimental period of 15 (small) to 21 days (medium and large).

Table 2. Feeding rate of carbohydrate, protein and fat by *M. cupanus* fed on different combinations of liver and *S. maxima*.

Food combination	Carbohydrate			Protein			Fat		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
100% liver	10.53	7.94	4.99	20.88	15.72	9.88	12.88	9.69	6.09
75% liver	9.46	7.43	4.69	17.96	12.95	8.21	11.02	8.04	5.10
25% algae									
50% liver	8.18	6.71	3.99	13.20	10.85	6.61	8.13	6.78	4.09
50% algae									
25% liver	6.93	5.13	4.20	7.60	5.91	4.82	4.53	3.54	2.89
75% algae									
100% algae	8.89	3.71	4.04	2.58	1.10	1.18	1.06	0.45	0.49

the small weight group receiving 50%A : 50%L, (ii) fat and protein conversion rate was maximum in the small weight class receiving 100%L, (iii) in the group fed exclusively on algae, rate of loss of body protein/fat for maintenance increased with decreasing body weight and (iv) small weight class converted protein, fat and carbohydrate at a faster rate than the large weight class.

The experimental fish converted carbohydrate less or more efficiently at all the feed combinations (table 3). When the feed contained over 50% carbohydrate maximum conversion efficiency was observed in the large weight group and it increased with increasing body weight. Data obtained for the conversion efficiency of fat and protein was quite different from the value obtained for carbohydrate. In these cases maximum efficiency was observed in the small weight groups and it decreased with increasing body weight. In addition, test individuals receiving less than 50% liver did not convert

**Figure 3.** Rate of protein conversion by large, medium and small weight classes of *M. cupanus* exposed to animal, algal and combination feeding.

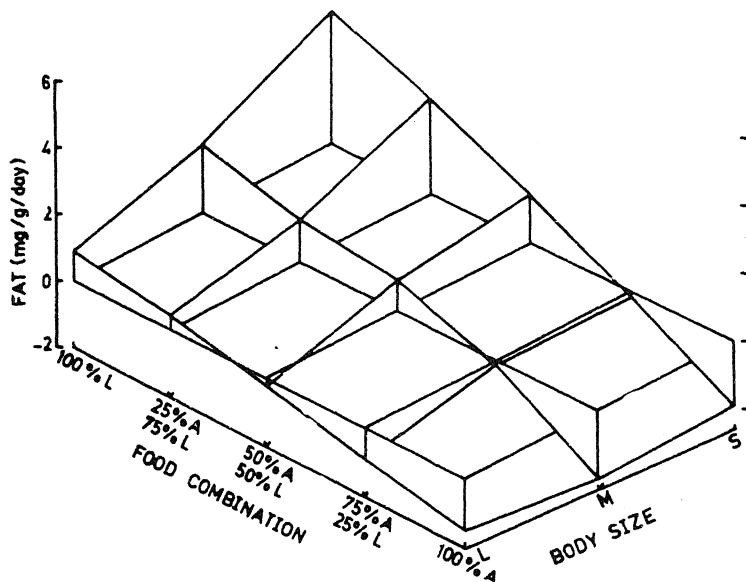


Figure 4. Rate of fat conversion by large, medium and small weight classes of *M. cupanus* exposed to animal, algal and combination feeding.

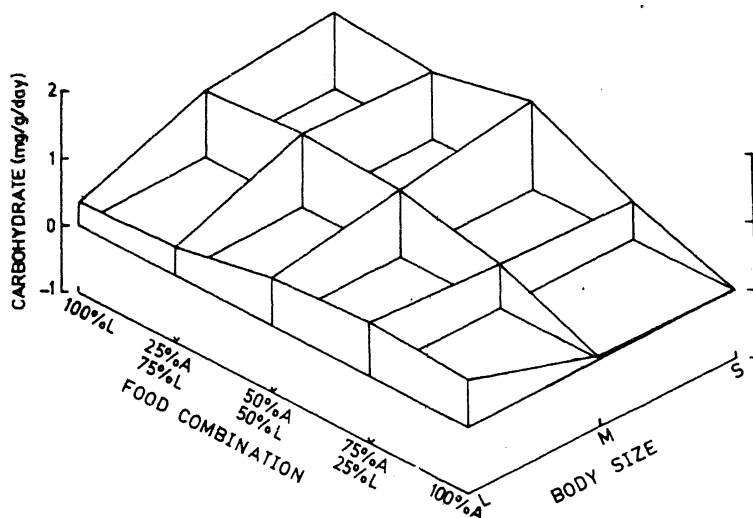


Figure 5. Rate of carbohydrate conversion by large, medium and small weight classes of *M. cupanus* exposed to animal, algal and combination feeding.

fat/protein. Another interesting observation is that *M. cupanus* converted fat more efficiently (26.5%) than protein (18.3% small weight group).

Table 3. Conversion efficiency of carbohydrate, protein and fat by *M. cupanus* fed on different combinations of liver and *S. maxima*.

Food combination	Carbohydrate			Protein			Fat		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
100% liver	9.45	13.22	8.59	18.29	14.44	13.01	26.55	21.20	17.36
75% liver 25% algae	8.92	15.20	10.76	13.71	8.75	10.81	22.17	15.61	10.39
50% liver 50% algae	13.64	16.21	22.19	10.91	7.47	7.48	15.40	13.33	—
25% liver 75% algae	6.79	12.74	23.51	1.00	—	5.16	—	—	—
100% algae	0.10	8.60	21.84	—	—	—	—	—	—

4. Discussion

Utilization of dietary protein, fat and carbohydrate by fish depends on the size (Steffens 1981), food quality and quantity (Papoutsoglou and Papoutsoglou, 1978) Austreng, 1981). In this study almost all the factors are tested to study the utilization of dietary constituents. The animal diet used in the present study contained 40.6% protein. Fischer (1972) reported a protein content in the animal feed as 51.6%. She used live tubificid worms as the animal food. Marian (1982) reported that tubificid worms are highly nutritious since they contain high protein (60%). Goat liver, used in the present study as the animal feed did not contain protein equivalent to the live prey. However, the protein content (14.6%) and fat content (6.0%) reported for the algae *S. maxima* are in par with those reported by Fischer (1972) for the plant feed *Lactuca sativa* (protein 15.3%; fat 5.8%). Available information on the feeding of algae or plant food by omnivorous fish revealed that the individuals are incapable of consuming enough algae to exhibit growth, to support maintenance and even resulted in the loss of body tissues (*Holocanthes bermudensis*; Menzel 1959; *Lepomis macrochirus*: Kitchell and Windell 1970; *Ctenopharyngodon idella*: Fischer 1973; *Tilapia mossambica*: Mathavan *et al* 1976; *M. cupanus*: Mathavan and Christopher 1980). The obvious reason is that these plant feeds contained the highest proportion of carbohydrate. The increased proportion of this constituent appears to reduce the feeding ability of the fish and/or it is not a palatable food with high feeding stimulants. Total food intake has been reduced to 5-10 times in algal fed group than the animal fed group (Fisher 1973; Mathavan and Christopher 1980).

In the present study total intake of carbohydrate, fat and protein were individually estimated both in the algal, liver and combination feed groups. It shows that carbohydrate consumption in the algal feeding group is reduced than in the liver feeding group. However, the reduction is not highly pronounced. On an average *M. cupanus* consumed 10 times more protein or 17 times more fat in the animal feeding group than the algal feeding group. It may be true that the increasing quantity of liver in the feed has resulted in continued stimulation of feeding and enabled the fish to maximise consumption. Fischer (1972) reported that there is a doubling of protein or fat consumption in *C. idella* exposed to tubifex worms than those exposed to a plant food.

Feeding the fish exclusively on algal diet resulted in negative conversion (loss of body weight) (Menzel 1959; Fischer 1972; Mathavan *et al* 1976; Mathavan and Christopher 1980). They failed to analyse the conversion of individual constituents of the feed. The present study shows that carbohydrate is converted more or less efficiently even when the animals are fed exclusively on algae. However, the trend obtained for fat and protein conversion shows that the fat serves as an initial source of energy during starvation or under feeding. It is clear from table 3 that large weight class receiving 50%A : 50%L shows negative conversion of fat, while all the weight classes converted protein exposed to the same feeding schedule. At 75%A : 25%L combination, all the test individuals used stored fat for maintenance while a certain percentage of protein was converted in the small and large weight classes. Critical analysis of the results show that feeding on insufficient quantity of food *M. cupanus* initially utilized stored fat and subsequently added stored protein for maintenance metabolism. This is supported by an earlier observation of Cowey and Sargent (1979) who showed that fish utilized considerable quantity of lipids as the source of metabolic energy to protect the structure and integrity of cellular membranes.

The observation that *ad libitum* fed *M. cupanus* converted fat more efficiently than protein is supported by the data recalculated from Huisman *et al* (1979) and Huisman

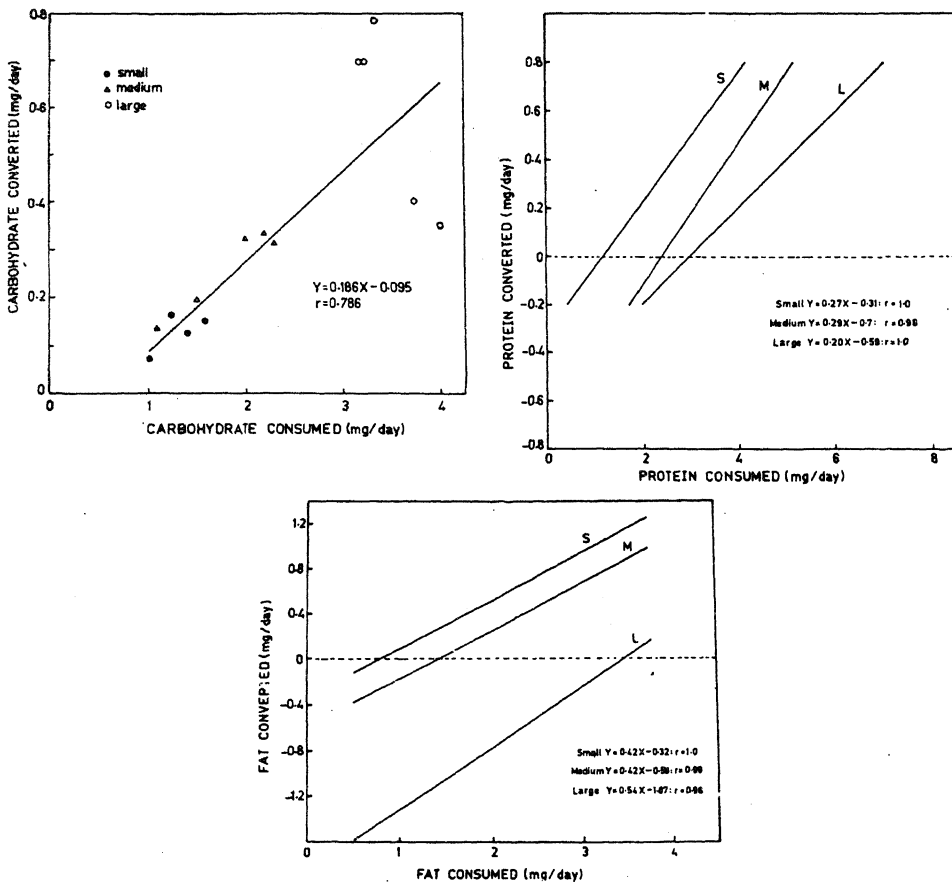


Figure 6. Regression to describe the relation between consumption and conversion (mg/day).

and Valentijn (1981) that *Cyprinus carpio* and *C. idella* fed on diets containing different proportions of fat and protein converted fat more efficiently than protein.

Conversion of carbohydrate, protein and fat (mg/day) was calculated and regressed against their respective consumption (mg/day) (figure 6a,b,c). For carbohydrate, regression was calculated pooling the data for different body weight (figure 6a). Consumption and conversion of carbohydrate increased with increasing body weight. Even at the low consumption of 1 mg carbohydrate/day, *M. cupanus* converted about 0.1 mg. In the case of protein and fat, when the consumption was less than 0.3% of the body weight, the large weight group oxidized stored fat or protein for maintenance. Both small and medium weight classes required fat consumption of about 0.6% of the body weight or 0.9% body weight of protein/day to retain the stored fat or protein in the body. When the consumption was below these values the fish lost different proportions of stored fat or protein for metabolism.

On correlating the consumption of protein or fat to conversion (mg/day) it became apparent that consuming 3.8 mg of fat/day the large, medium and small weight classes converted 0.2, 0.9 and 1.2 mg/day respectively (figure 6c) i.e. large, medium and small weight classes used about 96, 77 and 70% of the consumed fat for metabolism. In the case of protein, to convert about 0.8 mg/day small, medium and large weight classes consumed about 4, 5 and 7 mg protein/day respectively (figure 6). In other words they oxidized about 80 (small size), 84 (medium size) and 89% (large size) of the consumed protein for metabolism. On an average *M. cupanus* used about 82% of fat and 84% of protein for metabolism. Based on these observations it may be concluded that the fish *M. cupanus* utilized fat and protein more less equally for metabolism.

Acknowledgement

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Energetics of earthworm populations in tropical pastures from India

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Abstract. This paper reports our results of studies connected with the energy flow in earthworm populations in an upland and a lowland pasture where grazing by large herbivores were not allowed. The net annual energy input was more than double in the lowland pasture. Flooding during the rainy season caused heavy mortality and migration of worms in lowland pasture. Higher energy consumption through respiration in the upland pasture was due to difference in environmental conditions. Lower amount of secondary production (P) and higher amount of energy utilized in respiration (R) cause an increased R:P ratio in upland pasture. Energy channelization in both the sites has been represented in box type energy flow models. Management of these pasture lands depends on the understanding of the dynamics of energy flow in these systems.

Keywords. Earthworm; tropical pasture; energetics; primary production; secondary production; assimilation; energy flow model.

1. Introduction

Quantification of parameters in ecological energetic studies is important for the understanding of their relationships within the ecosystem. The ecosystems can be compared by energetic studies to understand their complexities. Earthworms dominate the biomass (> 80%) of invertebrates in grasslands and pasture ecosystems of the world (Watanabe 1975; Dash 1978; Senapati 1980). Their role as an important consumer and decomposer group have been elucidated by Darwin (1881); Satchell (1967); Dash and Patra (1977, 1979); Dash (1978); Lavelle (1977, 1978); Senapati *et al* (1980); Senapati and Dash (1982a). Macfadyen (1963) has calculated that 2/7 of the gross primary production is utilized by aboveground grazers, 1/7 is dissipated during plant respiration; the remaining part passes into the soil system. This together with the faeces and corpses of animals forms the energy base on which the pedofaunal and pedofloral decomposers in the soil operate. Dash and Patra (1977), Senapati and Dash (1982b) for the first time showed the energetic relationship of earthworms, a dominant consumer group in tropical pastures, with regard to primary production. Senapati and Dash (1981) worked out the effect of grazing on the energy flow mechanism in both the vegetation and earthworm components in tropical pastures. This paper compares the energetic relationship of the earthworm *Lampito mauritii*, Kinberg dominant in a lowland pasture with that of *Octochaetona surensis* (Michaelsen) dominant in an upland pasture of Orissa.

2. Study sites

Lowland site is situated at Berhampur and the upland site at Sambalpur, Orissa, India. Both the sites are located in 19-22°N latitude and 83-85°E longitude and are protected from large herbivore grazing. Grasses accounted more than 80% (on the basis of

Table 1. Biological and physical characteristic of the study sites.

Characteristics	Berhampur lowland pasture	Sambalpur Upland pasture
Dominant Grass species:	<i>Cynodon dactylon</i> , <i>Hygrohiza</i> sp.	<i>Cynodon dactylon</i> , Pers <i>Cynodon</i> sp. <i>Eragrostis amabilis</i> , (Wight & Arn)
Earthworm species:	<i>Lampito mauritii</i> , Kinberg <i>Ocnerodrilus occidentalis</i> . Eisen	<i>Drawida calebi</i> , Gates <i>D. willsi</i> , Michaelsen <i>L. mauritii</i> <i>Octochaetona surensis</i> (Michaelsen) <i>O. occidentalis</i>
Air temperature		
(Mean (maximum) seasonal temp. in °C at 30 cm above soil surface)		
Summer	34 (42)	33 (46)
Rainy	28 (33)	29 (37)
Winter	24 (30)	23 (33)
Soil temperature		
(Mean (maximum) seasonal temp. in °C at 5 cm deep)		
Summer	30 (38)	35 (40)
Rainy	28 (30)	28 (31)
Winter	22 (25)	23 (29)
Soil moisture		
(Mean (maximum) seasonal moisture in g% at 10 cm deep)		
Summer	13 (16)	7 (12)
Rainy	30 (40)	15 (17)
Winter	23 (26)	12 (13)
Rain fall		
(Seasonal total rain fall in cm)		
Summer	7	8
Rainy	84	122
Winter	1	5

biomass) of the vegetation within the study sites. Details of biological and physical characteristics of the study sites are given in table 1. On the basis of biomass, *L. mauritii* and *O. surensis* earthworms were dominant (>50%) in the lowland and upland sites respectively. Both the sites experience a dry summer (March-mid June) followed by heavy monsoon rains (mid-June-October) and a mild winter (November-February). The pH of soil of both the sites was slightly acidic (6.5-6.8).

3. Material and methods

3.1 Primary production

Vegetation biomass of each component (above ground parts and below ground parts) was estimated in g dry wt/m²/month. The net primary production (NP) has been estimated by utilizing the annual incremental biomass harvest method (Sims and Singh 1971; Senapati and Dash 1981).

3.2 Secondary production

Secondary production (*P*) is defined as the total amount of tissue substance produced over a particular time period (generally one year) irrespective of whether it has survived to the end of that period or not (Macfadyen 1967). Energy budget equation given by Petrusewicz and Macfadyen (1970) can be written as:

$$C = A + FU, \quad A = P + R, \quad P = E + \Delta B$$

where *FU*: rejecta, faeces and urine; *A*: assimilation; ΔB : change in biomass (growth and reproduction); *C*: consumption; *E*: Elimination (the biomass of the individual that have died or been killed); *P*: production; *R*: respiration.

In the present investigation all the parameters have been estimated except direct quantification of consumption.

Production has been estimated by biomass difference method (Dash and Patra 1977; Senapati and Dash 1981). Changes in number and biomass of worms of each age group showed the loss or gain of worms. Growth and mortality were thus calculated from the gain and loss of biomass respectively. Particular amount of worm biomass lost by predation, parasitism etc has not been quantified separately. Details of cocoon production has been discussed by Dash and Senapati (1980).

Oxygen uptake by different age class and size groups of different species of earthworms was measured in the laboratory by Winkler's gaseous displacement method as described in Welch (1948). Total amount of energy spent through respiration has been extrapolated for the respective study sites considering the prevalent field soil temperature. Excretion of mucus protein, free amino-acids, ammonia and urea were determined under laboratory conditions at average field soil temperature by spectrophotometric method as described by Kaplan (1969) for ammonia and urea, by Moore and Stein (1948) for total free amino-acids, by Lowry *et al* (1951) for mucus protein.

Energetic conversion of different materials has been made by utilizing standard values as:

Materials	Energy content (kJ/g dry wt)	Reference
Plant tissue (grass)	16.62	Jain and Mishra (1972)
Earthworm tissue	19.34	Golley (1961)
Mucus protein	16.74	Satchell (1971)
Free amino acid	16.74	Satchell (1971)
Ammonia	16.95	Barfield and Solomon (1972)
Urea	10.47	Satchell (1971)
Oxygen	20.10	Engelmann (1961)

4. Results

Figure 1 shows a box type flow model of energy in the earthworm populations in the lowland and upland pastures. The annual net primary production was about 32148 kJ and 16037 kJ/m² in the lowland and upland pasture, respectively. Average monthly live earthworm biomass in the lowland was 32 g (8 g dry wt)/m² and 56 g (11 g dry wt)/m² in the upland pasture sites. Oxygen consumption of earthworm population amounted to 60 l in the lowland and 70 l/m²/year in the upland pasture. The energy equivalent of this oxygen consumption would amount to 1206 kJ and 1440 kJ/m²/year, respectively. This respiratory activity does not take into account the motor activity such as burrowing or feeding. Annual energy output in excretion including mucus production was 2378 kJ and 573 kJ/m² in the lowland and upland site, respectively (figure 1). Earthworm secondary production amounted to 678 kJ/m²/year in the lowland and 511 kJ/m²/year in the upland site. However secondary production value in the lowland site does not include the tissue utilized in

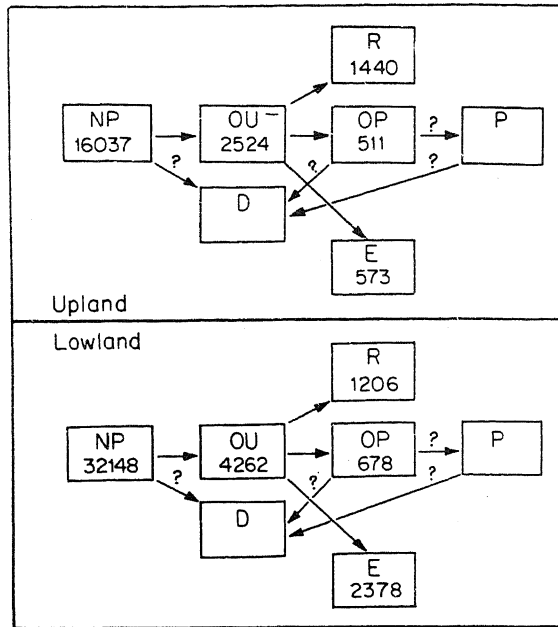


Figure 1. Box type flow model of energy in the earthworm populations in lowland and upland pasture.

Abbreviations: NPP, Net primary production; OP, Oligochaete Secondary Production; OU, Oligochaete Utilization; E, Oligochaete Excretion; R, Oligochaete Respiration; P, Predators and Parasites; ?, Parameters not estimated. All energy values in kJ/m²/yr.

reproduction, *i.e.* in cocoon formation. In the upland site, tissue utilized in cocoon production contributed 7% of the secondary production. Worm tissue loss due to mortality contributed 60% and 50% of the secondary production in the lowland and upland site, respectively. Turnover rate in the oligochaete component was about 4.5 and 2.5 in the lowland and upland pasture respectively. Energy loss through excretion in earthworms occur in two ways *i.e.* mucus production and nephridial excretion. This is also part of the assimilated energy. But for convenience, assimilation has been defined in this work as the sum of secondary production and respiration. Total sum of assimilation and excretion has been considered as total oligochaete utilization. Total annual assimilated energy (secondary production + respiration) was 1884 kJ and 1951 kJ in the lowland and upland pastures respectively (figure 1). Estimation of production efficiency (ratio of secondary production to assimilation $\times 100$) comes to 36% and 26% in the lowland and upland pastures, respectively. Ratio of respiration and secondary production was 2 and 3 in the lowland and upland sites, respectively. Total annual energy utilization $\approx$ energy consumption) in earthworms amounted to 4261 kJ in the lowland site and 2524 kJ/m² in the upland site. Earthworm utilization is approximately 13% and 16% of the net annual energy input in the lowland and upland pastures, respectively. Earthworms spent 64% and 74% of the assimilated energy in respiratory metabolism in the lowland and upland site, respectively. These estimated values of energy utilization by earthworms represents only assimilated energy; actual consumption is greater.

5. Discussion

Among the oligochaetes, Megascolecids make a major contribution to the oligochaete production in some tropical pastures of India (Dash 1978). In both the lowland and upland sites, in the absence of large herbivore grazers, all the energy input from the vegetation component was available for soil microflora and fauna. Net primary production was more than double in the lowland pasture. This in comparison with the upland pasture may be attributed to high soil moisture content (table 1). The interesting part of the result is that although in the lowland pasture, the monthly average earthworm biomass was 38% lower than the upland pasture, the earthworm secondary production was around 33% higher. The causes responsible for this difference may be heavy mortality and migration of worms during flooding in rainy season in the lowland site (table 1, figure 1). It has been estimated that the mortality tissue was around 10% higher in the lowland site. Earthworm cocoon production has not been studied in the lowland pasture, which would have increased the secondary production by around 7%. Dash and Senapati (1980) found that a moisture condition of around 10-20 g% with soil temperature of 20-30°C, is the most favourable range for tropical earthworms. They have also found that in complete hydric condition, cocoons never hatch. They have shown that at lower moisture (< 10 g%) and higher soil temperature (> 30°C) some earthworms like *O. surensis*, form diapause coils to withstand the summer stress. Although the soil temperature condition remained almost the same in both the sites, there was higher soil moisture content in lowland pasture than the upland (table 1). During summer the mean average soil moisture content at 10 cm depth was 13 g% and 7 g% in the lowland and upland pasture soil, respectively. This along with the higher summer temperature (> 30°C) might be the cause for higher energy consumption through respiration, in the upland pasture (about 19% higher) (figure 1).

In energetic studies ratio of respiration (R) and production (P) is another parameter to represent metabolic status. The R/P ratio reported by Satchell (1971) and Nowak (1975) was 2 and by Hutchinson and King (1979) was about 3 for large oligochaetes. The present reported value of 2 in lowland and 3 in upland pasture agree with these findings. Lower amount of secondary production (P) and higher amount of energy utilized in respiration (R) are the causes for increased R/P ratio in the upland pasture. It is evident that with increased tissue mortality in the lowland site, the loss is compensated with higher tissue growth efficiency (Odum 1959) (38.5% higher than upland pasture). This study gives further evidence in favour of the generalization made that high environmental stress usually results in repair (Bouche 1971; Lavelle 1978, 1980). The present tissue growth efficiency (26% for upland, 36% for lowland) is very close to previous findings. Satchell (1971) and Hutchinson and King (1979) have reported a tissue growth efficiency value from 27.5 to 34% for earthworms. The turnover rate (ratio of monthly average biomass to annual production) of earthworm tissue is almost double in the lowland pasture than the upland. However because of the methodological difference in the study of excreted energy loss, it was not possible to compare these values. Considering the total energy utilization it is estimated that earthworms in the upland pasture utilize more portions (16%) of the energy input than in the lowland pasture (13%).

From the energy utilization values oligochaetae seem to be very important in the decomposer system of both upland and lowland pastures. Management of these pasture lands depends on efficient ecological manipulation of these organisms.

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Effect of tranquillisers on the reproduction of a freshwater leech, *Poecilobdella viridis* (Blanchard)

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Abstract. Effects of two well-known tranquillisers, reserpine and chlorpromazine on the reproduction of *P. viridis* were investigated. There was a significant decrease on the ovarian index after reserpine (RSP) injection, whereas, chlorpromazine (CPZ) did not induce any change. But chlorpromazine has a profound effect on the testis index. Surprisingly, injection of the mixture of RSP + CPZ induced a significant decrease in the ovarian and testis indices. Besides gonad index, the tranquillizer effect on histological profile of gonads was also studied.

Keywords. Tranquillisers; *Poecilobdella viridis*; reserpine; chlorpromazine.

1. Introduction

It is well known that a number of drugs and pharmacological agents provoke changes in the physiological functions of animals by affecting directly or indirectly the neuroendocrine centres of both vertebrates (Sarkar *et al* 1977) and invertebrates (Masner *et al* 1976; Hanumante *et al* 1979 a,b; Kulkarni *et al* 1982). The pharmacological effect of reserpine, a well-known tranquilliser depresses egg fertility in turkey hens (Arend *et al* 1972) and decreases the weight of ovary and oviduct in the female Indian koel (Sarkar *et al* 1977). In invertebrates reserpine is reported to decrease the egg production in insects (Raina 1974), whereas in the crab, *Scylla serrata*, its administration increased oocyte diameter and initiated the oocyte maturation from pre-vitellogenic to vitellogenic stages (Farooqui 1980). Another tranquilliser, chlorpromazine is also known to have similar effect as that of reserpine but differs in the way of action (Hess 1954). It has been reported that the chlorpromazine injection induced changes in the cerebral ganglion neurosecretory activity of a freshwater bivalve, *Indonaiia coeruleus* (Hanumante 1979b). The injection of chlorpromazine was reported to have no effect on the gonads of *S. serrata* (Farooqui 1980).

Since there is no convincing report so far regarding the effect of these important tranquillisers on the reproductive activities of annelids, the present study has been undertaken, using a locally available hirudid annelid, *P. viridis*.

2. Material and methods

The leeches, *P. viridis*, were collected randomly from the freshwater ponds around Aurangabad. After 72 hr of acclimation to the prevailing laboratory conditions, the leeches having no clitellum and approximately the same weight were sorted out and divided into five groups of ten each.

- Group I : Untreated initial control (IC)
 Group II : Simultaneous control (SC) injected with distilled water (50 μ l/leech).
 Group III : Reserpinised (RSP): Leeches administered with reserpine (RSP 50 μ g = 50 μ l/leech)
 Group IV : Chlorpromazinised (CPZ) Leeches injected with chlorpromazine (CPZ : 50 μ g = 50 μ l/leech).
 Group V : Mixture of RSP + CPZ Leeches injected with equal volumes of the mixture of reserpine and chlorpromazine (RSP + CPZ = 25 μ g + 25 μ g = 50 μ l/leech)

Initial control leeches were dissected at the start of the experiment and their gonads were fixed in aqueous Bouin's fluid after calculating the gonad index. Whereas the remaining groups of leeches were sacrificed after 15 days of their respective treatments. Ovary and testis were dissected and fixed in the fixative after calculating the ovarian index (OI) and testis index (TI) using the formula of Farman Farmaian *et al* (1958). Fixed tissues were processed for histological preparations using the method of Kulkarni *et al* (1978). Wherever necessary data were statistically analysed using Student *t* test (Ostle 1966).

3. Observations and results

3.1 Ovarian and testis indices

The results presented in table 1 clearly show that there was a significant ($P < 0.05$) decrease in the ovarian index after reserpine injection. However chlorpromazine induced a significant ($P < 0.05$) decrease in the testis index. But reserpine and chlorpromazine did not induce any change in the testis and ovarian indices respectively. It was however noticed that the mixture of reserpine and chlorpromazine caused a significant ($P < 0.01$) decrease in both ovarian as well as testis index.

3.2 Histo-morphological changes

Depending upon the histological studies, Kulkarni *et al* (1978) have already characterized and classified the different stages of ovarian development (4 stages) and spermatogenesis (4 stages) in *P. viridis*.

Table 1. Showing the effect of tranquillizers on the gonadal index of *P. viridis*.

Treatment	Ovarian index	Testicular index
Initial control	1.7331 $\pm$ 0.02	31.195 $\pm$ 2.41
Simultaneous control	1.660 $\pm$ 0.03	30.33 $\pm$ 2.22
Reserpine (50 μ g/leech)	0.976 $\pm$ 0.004	30.6569 $\pm$ 2.32
Chlorpromazine (50 μ g/leech)	1.534 $\pm$ 0.01	27.423 $\pm$ 0.09
Mixture of Reserpine + Chlorpromazine (50 μ g/leech)	0.883 $\pm$ 0.002	25.905 $\pm$ 1.01

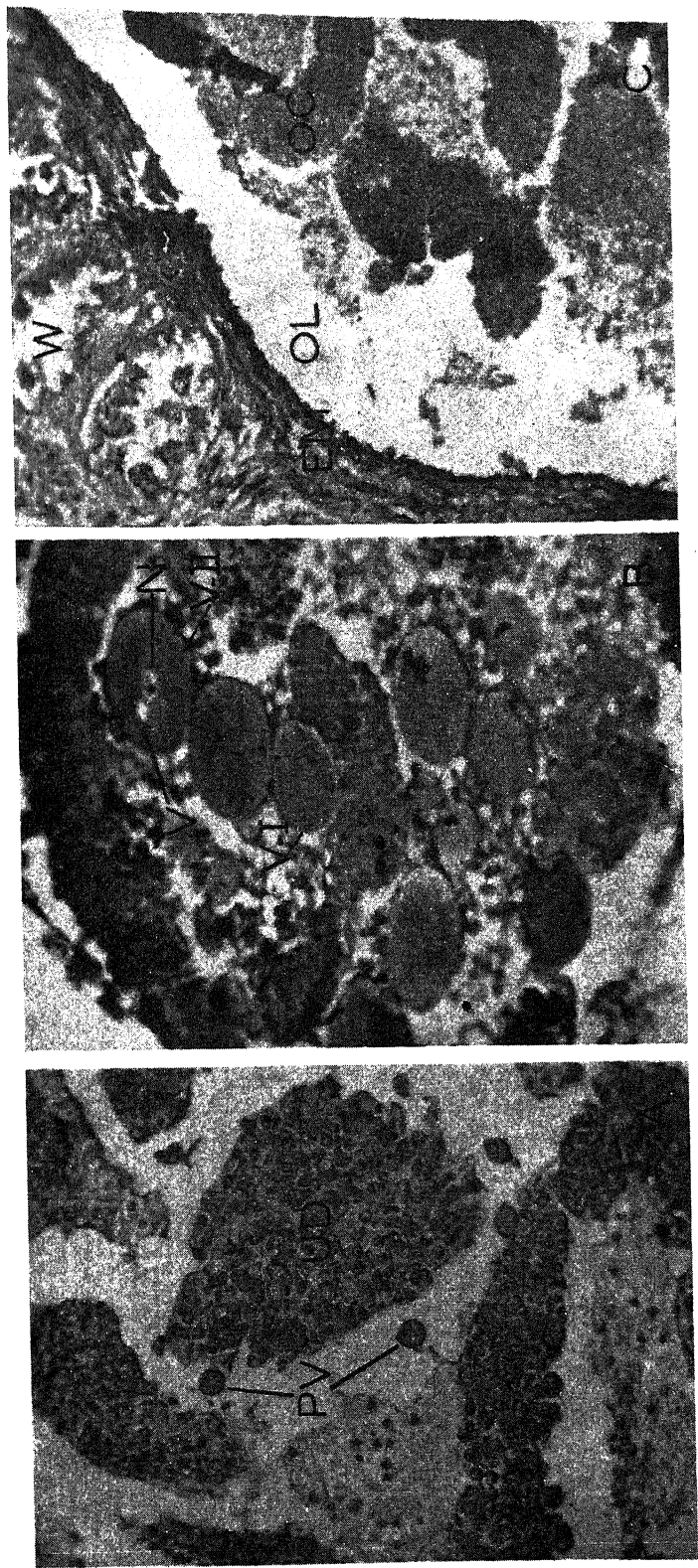


Figure 1. (See captions in p. 326)

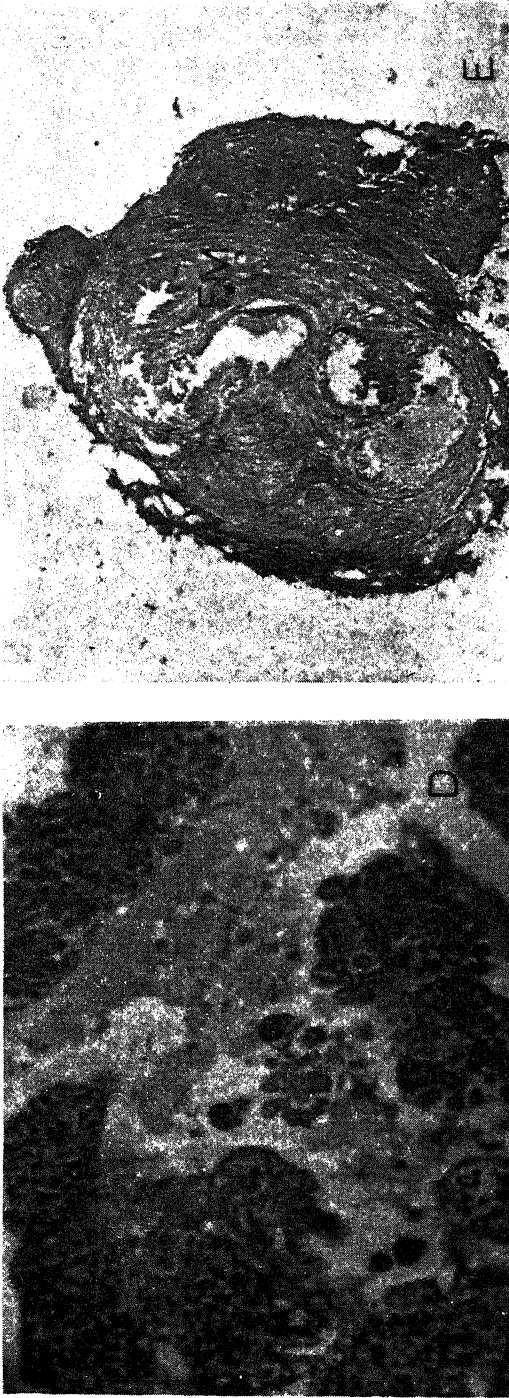


Figure 1. Effect of tranquilizers on the ovary of *P. viridis*. A. TS of ovary of the untreated initial control leeches. Undifferentiated (UD) and previtellogenic (PV) stages are seen $\times 150$. B. TS of ovary of the simultaneous control leeches. Vitellogenic-I (V-I) and vitellogenic II (V-II) stages are seen $\times 150$. (N = Nucleus; V = Vacuole). C. TS of ovary of the leeches treated with reserpine for 15 days. Note the regressing ovarian cords (OC) into the ovarian lumen (OL). White vacuole or packet (W) like structures are seen in the mesodermal area of ovarian wall $\times 150$. (Em = Endometrial layer of ovarian wall). D. TS of ovary of the chlorpromazine injected leeches. Note that there was no profound effect and histological profile is almost mirroring the profile of A $\times 150$. E. TS of ovary of the leeches administered with mixture of RSP + CPZ. Note that ovarian lumen is almost completely invaded with endometrial tissue of ovarian wall. No stages of ovarian development are visible $\times 60$.

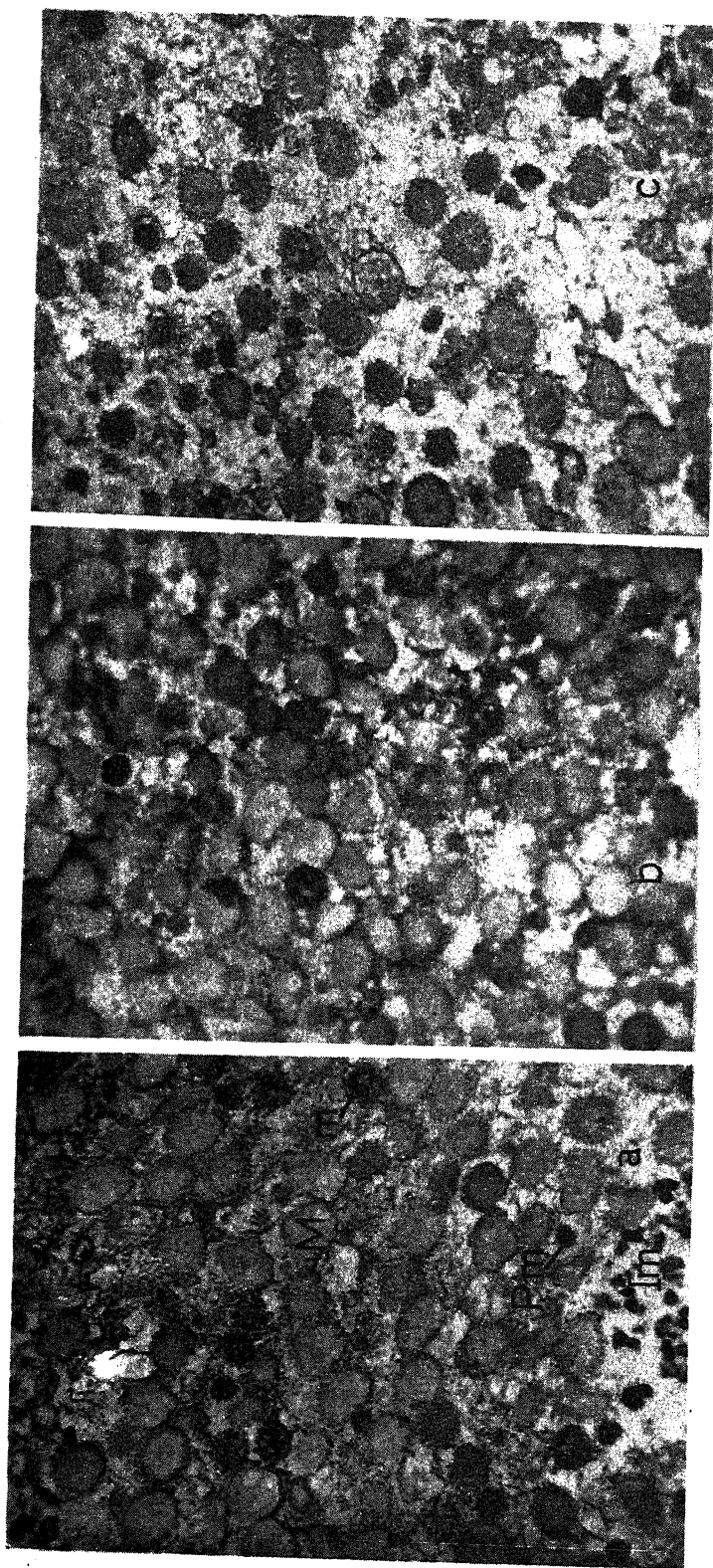


Figure 2. (See captions in p. 328)

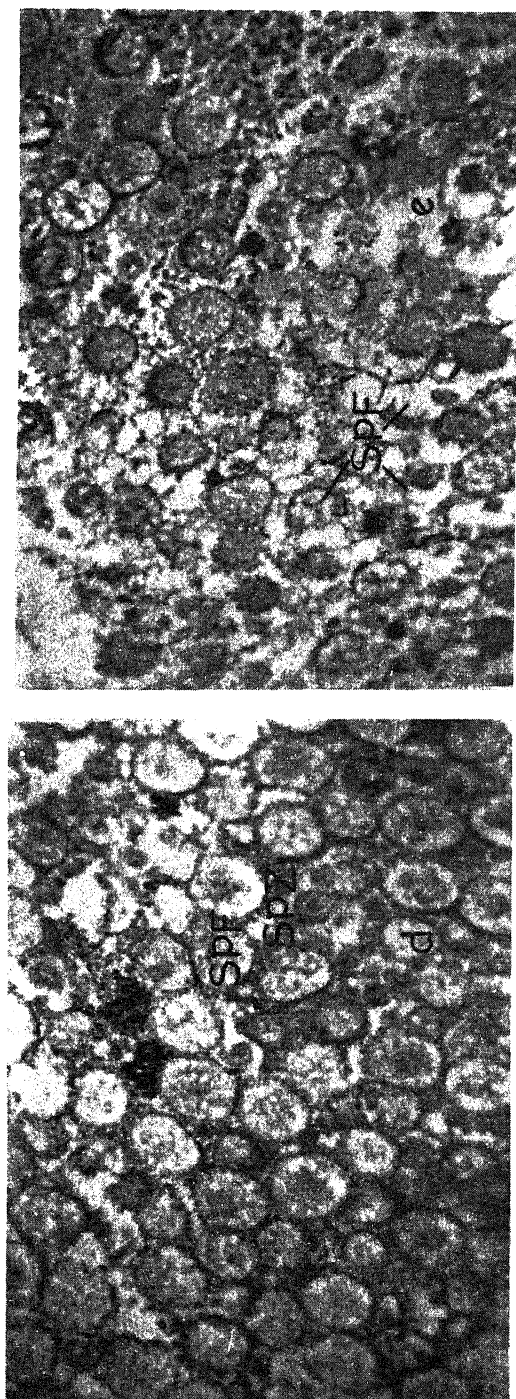


Figure 2. Effect of tranquilizers on the testis of *P. viridis*. **a.** TS of testis of untreated initial control leeches. Note the immature (Im), premature (Pm), maturing (m) and mature (M) stages $\times 150$. **b.** TS of testis of simultaneous control leeches. Note the greater number of mature spermatogenic follicles $\times 150$. **c.** TS of testis of reserpinized leeches. No change in the profile of spermatogenic follicles was visible except reduction in the total count of stages $\times 150$. **d.** TS of testis of the leeches injected with chlorpromazine. Severe emptying of spermatogenic follicle (SPF) was seen. Regressing spermatogenic follicles are noticed with the spermatozoa occurred at follicle wall $\times 150$. **e.** TS of testis of the leeches treated with the mixture of RSP + CPZ. Drastic regression of spermatogenic follicles is visible $\times 150$.

Table 2. Effect of tranquillizers on the histological profile of gonads in *P. viridis*.

	% stages of ovarian development (average size of the oocyte in μm)				% stages of testis development			
	Undifferentiated	Previtellogenic	Vitellogenic-I	Vitellogenic-II	Immature	Premature	Maturing	Matured
IC	93 (17.55 $\pm$ 2.2)	5 (23.4 $\pm$ 2.1)	2 (35.1 $\pm$ 1.6)	—	17	13	25	45
SC	—	22 (23.4 $\pm$ 1.8)	26 (35.1 $\pm$ 2.6)	52 (70.2 $\pm$ 6.4)	3	8	6	83
RSP	Drastic arrest of ovarian maturation. Many vacuoles or white packet like structures observed in the mesodermal layer of ovarian wall showing degeneration and atrophy				3	33	30	34
CPZ	97 (17.55 $\pm$ 2.2)	3 (23.4 $\pm$ 1.8)	—	—	There was a complete emptying of spermatozoa from spermatogenic follicle. Spermatozoa were found to migrate towards the spermatogenic follicle wall. Spermatogenic follicle was in advanced regression stage.			
RSP + CPZ	There was a profound change in the ovarian structure. Ovarian lumen invaded endometrial tissues and no stages of ovarian development were encountered				There was a significant decrease in the count of spermatogenic stages, showing high degree of degeneration. Many amoebocyte like cells and white patches were seen in testicular follicle.			

3.2 a. *Ovary*: Histological observations show that the ovary of initial control leeches mainly has small oogonia and few pre-vitellogenic stages (table 2, figure 1A). Ovaries of simultaneous control leeches had more advanced stages (such as enlarged oocytes and vitellogenic II stages) of ovarian development (table 2, figure 1B). But figure 1C indicated clearly that there was a drastic arrest of ovarian maturation after reserpine injection (figure 1C). Many vacuole-like structures were encountered in the mesodermal layer of ovarian wall indicating the start of degeneration and atrophy of the ovary. But chlorpromazine injection had no effect, either inhibitory or stimulatory, on the ovarian maturation (table 2, figure 1D). The mixture of RSP and CPZ induced very profound change in the ovarian structure itself (figure 1E). The ovarian lumen was almost completely invaded with endometrial tissues and no stages of ovarian development were seen.

3.2 b. *Testis*: Histological features of the initial control testis showed that testis follicle contain spermatogenic follicles, mainly in premature, maturing and matured stages of spermatogenesis (table 2, figure 2a). Testis of simultaneous control leeches had mostly mature and few maturing stages (table 2, figure 2b). Surprisingly, reserpine injection did not induce any significant change in the testis except that there was a slight decrease in the total count of maturing and mature spermatogenic stages (table 2, figure 2c). Chlorpromazine had profound effect only on the maturing and mature spermatogenic stages (figure 2d). There was complete emptying of the mature spermatogenic follicle and the spermatozoa were found migrated towards spermatogenic follicle wall and accrued there. Spermatogenic follicles were seen in almost regression stage. Injection of the mixture of RSP + CPZ had a significant effect on the spermatogenesis. There was a drastic decrease in the total count of spermatogenic stages. Maturing and mature stages showed higher degree of degeneration. Many amoebocytes were seen in testicular follicles and white patches in the spermatogenic follicles (figure 2e).

4. Discussion

Numerous drugs and chemicals induce significant changes in the neurosecretory profile of both vertebrates and invertebrates, which in turn affects the physiological functions of animals. The data from table 1 show that the ovarian and testis indices of *P. viridis* decreased respectively after injecting reserpine and chlorpromazine separately. Histomorphological features of ovaries injected with reserpine and testis injected with chlorpromazine showed severe regression. These results indicated that reserpine and chlorpromazine have drastic but different pharmacological effects on the gonads of *P. viridis*.

It is well known that pharmacologically reserpine has a neuroleptic effect causing the release of a biogenic monoamine i.e. 5-hydroxytryptamine (5-HT) from the nervous tissues and chlorpromazine has an effect of blocking the release of noradrenaline from nervous tissues of vertebrates and invertebrates (Farooqui 1980). Earlier it was reported that 5-HT when injected into *P. viridis* interferes with the neurosecretory activity and has a traumatic effect on A type neurosecretory cells of brain (Kulkarni *et al* 1980), which are speculated to secrete gonadotrophic hormone (Nagabhushanam and Kulkarni 1980). Since a profound regression of ovarian structure was found in the present study after reserpine injection, it is quite possible that the released 5-HT might have interfered with the gonadotropin release, which is essential for normal oogenesis (Nagabhushanam and Kulkarni 1980) and maintenance

of the gonad (Mill 1978). Chlorpromazine was found to affect only the spermatogenesis since it is pharmacologically known to block the release of nor-adrenaline. It is speculated that this nor-adrenaline might be essential to release the essential gonadotropin neurohormone from the brain, which may stimulate spermatogenesis and maintain the spermatogenic follicle. Unfortunately no report is available so far either supporting or contradicting our later speculation. It is difficult to explain why there was drastic regression in gonads after injecting the mixture of RSP+CPZ in *P. viridis*. It is expected that this possibly may be a non-specific toxic effect on the target tissues causing them to degenerate. Further work is in progress to study the regression.

Acknowledgements

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Wing microsculpturing in the small house cockroach, *Supella longipalpa* (Dictyoptera, Blattidae)

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Abstract. Elytra and wings of *Supella longipalpa* (Fabricius) show sexual dimorphism in size, being well developed in males and less so in females. The following eight types of microsculpturing elements (including hairs) occur on both the upper and lower surfaces, varying in size (0.5-100 μm) and density (a few to 1000/ mm^2): Pimples, papillae, rods, microsetae, hairs (setae or macrotrichia of authors), polygons, parallel ridges and an irregular reticulum. The nomenclature adopted is mainly based on termites where these structures have been studied in great detail. Comparison is made with the Isoptera, Embioptera and Zoraptera. The biological and ecological consequences of microsculpturing are discussed.

Keywords. Wing microsculpturing; cockroach; *Supella longipalpa*.

1. Introduction

In recent years, several kinds of cuticular microsculpturings (in addition to hairs) have been found on the wings of a number of lower insect orders. The most remarkable in this respect are the termites (Isoptera) in which at least seven different types of discrete sculpturings have been recorded, varying greatly in shape, size and density. They range in size from *ca.* 0.5 - 23 μm , in shape from thin rods to finger-like or thorny papillae, tubercles, arrowheads, pimples and micrasters (with 1-8 arms), and in density from about 300 - 13000/ mm^2 (Roonwal 1977, 1981, and in press a,b); Roonwal *et al* 1967-1979, *vide* the references). In Embioptera (Roonwal and Rathore (in press)) three types of short to long 'hair-like' structures have been found, *viz.* macrosimitars, macrotrichia and microtrichia, with moderate to fairly high densities. In Zoraptera only short and long hairs are present (Roonwal in press b).

Since these orders are close to the cockroaches or Blattoidea (termites and cockroaches are believed to have arisen from a common Protoblattoid stock), and since no such structures appear to have been described in cockroaches so far except hairs (Miall and Denny 1886; Rehn 1951; Richards and Davies 1977; Chapman 1973; Bell and Adiyodi 1982), it was considered desirable to study wing microsculpturing in cockroaches. Lefeuvre (1969) mentions some sensillae on fore and hind wings of *Blaberus craniifer*.

In this paper microsculpturing in the commonly available small house cockroach, *Supella longipalpa* (Fabricius) (family Blattidae*, subfamily Pseudomopinae, tribe Supellini), is described.

* The classification of Rehn (1951) is followed. In another system, *Supella* is assigned to the family Blatellidae.

2. Material and methods

Samples of *Supella longipalpa* were obtained from kitchens at Jodhpur. It is fairly widespread in India and has also been reported from Punjab (Sandhu and Sohi 1981). Both elytra and wings were studied in glycerine mounts, which, as in termites, gave better definition of microsculpturing than permanent Canada balsam mounts. Densities were determined from camera lucida drawings. Nearly identical microsculpturing occurs on the upper and lower wing surfaces; the dorsal surface is described here.

3. Results

3.1. General

Elytra and wings show sexual dimorphism, being well-developed in males (figure 1) and less so in females (figure 2). On the basis of microsculpturing, each structure is divisible into several regions (vide infra).

3.2. Types of microstructures

The eight types of microstructures found in *Supella longipalpa* are described below.

3.2 (i) *Pimpules*: Minute, rounded or papillate, glassy, refractile structures occurring on the membrane in considerable density (ca. 1000/mm²); diameter 1.5 - 2.5 μ m.

3.2 (ii) *Papillae*: Small, thorny structures without a basal head (cf. microsetae). Size 0.5 μ m x 2 μ m. Rare

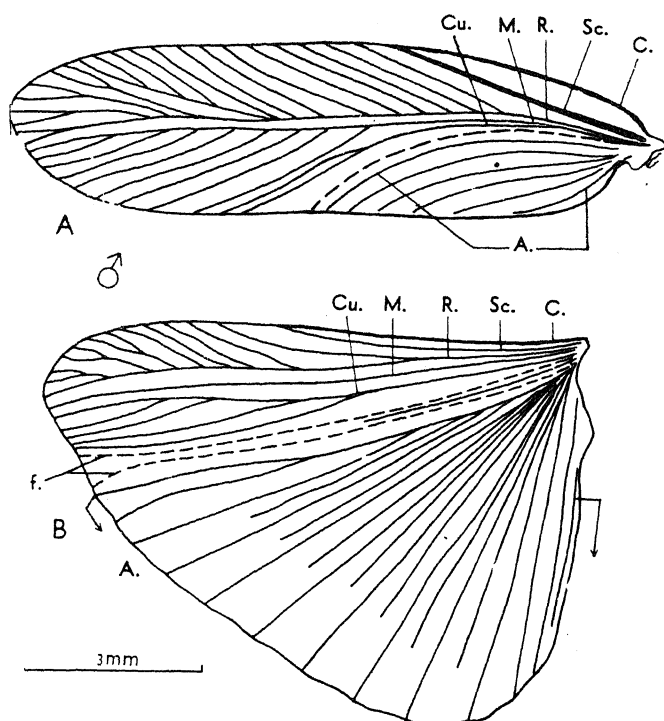


Figure 1. *Supella longipalpa*, male. **A.** left elytron **B.** left wing, to show venation (nomenclature based on Rehn 1951). (A, anals; C, costa; Cu, cubitus; f, fold, M, media; R, radius; Sc, subcosta).

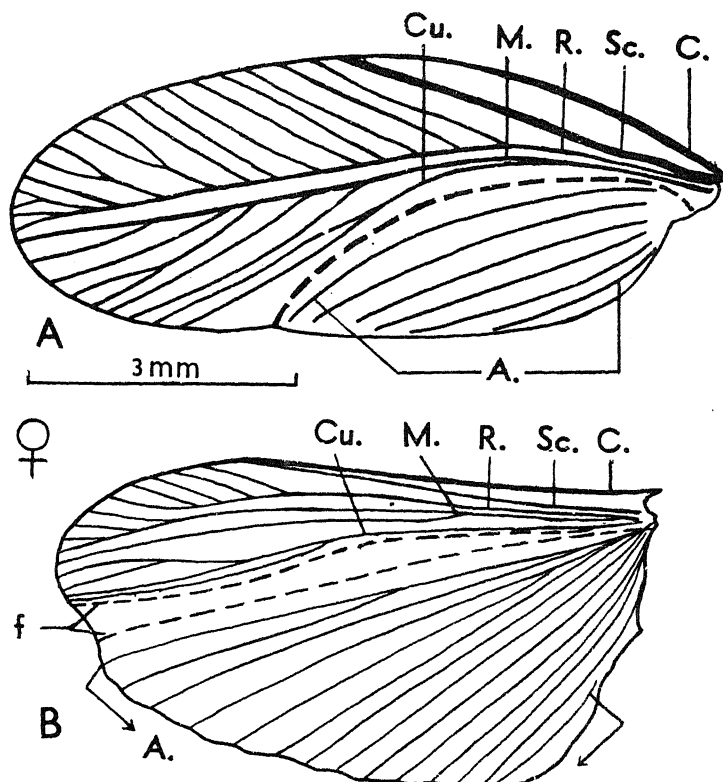


Figure 2. *Supella longipalpa*, female. A. Left elytron B. left wing, to show venation (nomenclature based on Rehn 1951). Abbreviations as in figure 1.

3.2 (iii) *Rods*: Small, thin or thick, rod-like bodies; substraight, crescentic or V-shaped. Length 10-15 μm ; density 310/ mm^2 . Usually thinner on veins than on membrane.

3.2 (iv) *Microsetae*: Short, thick, stubby, nail-like structures, with a broad, rounded basal head and a short, sharply pointed shaft. Length 7-14 μm ; density 300-400/ mm^2 .

3.2(v) *Hairs (setae)*: Hair-like structures with a basal rounded platelet and a longish, pointed shaft. Length 35-100 μm . Few and scattered.

3.2 (vi) *Polygons*: A pattern of adjacent polygonal areas enclosed by fine lines. Usually with 5 sides, sometimes subrectangular. Polygons 5-9 μm wide, 26-33 μm long.

3.2 (vii) *Parallel lines*: A pattern of fine parallel lines, 8-17 μm apart.

3.2 (viii) *Reticulum*: An irregular, indefinable reticulum of fine lines (probably ridges).

3.3. Microsculpturing in males

3.3a. *Elytra*: Length 11 mm, maximum width 3 mm. surface divisible into 4 regions (figure 3):

3.3a(i) *region no. 1* (figure 4 A, B): A small region at the base. Densely covered all over with minute pimpules (diameter 1.5 - 2.5 μm , density ca 1000/ mm^2).

3.3a(ii) *region no. 2* (figure 4 C, D): A longish region at the anterior margin. Covered

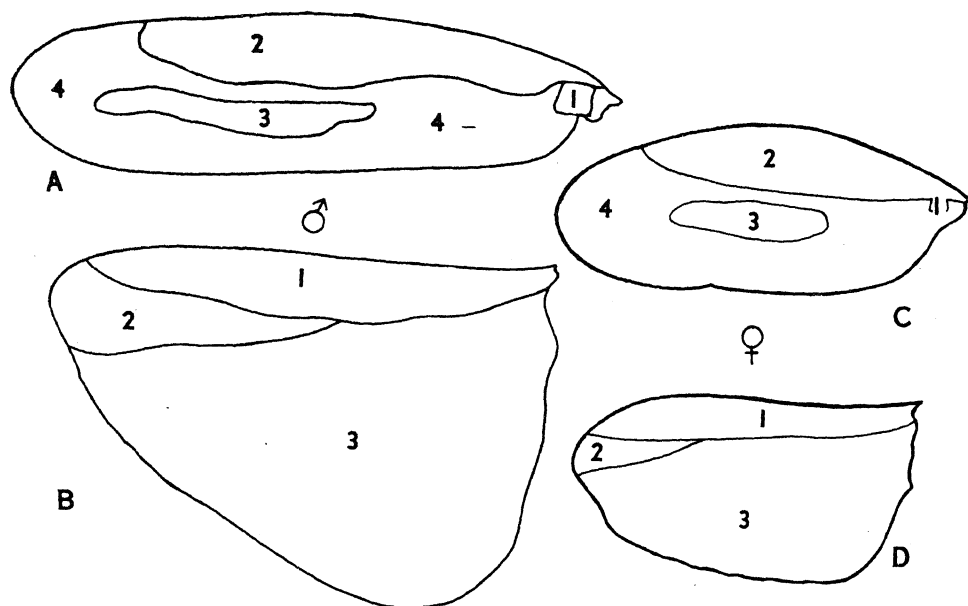


Figure 3. *Supella longipalpa*. Outlines of left elytra and wings, to show regions of different types of microsculpturing; region Nos. 1-4 in elytra and 1-3 in wings. A, B. Male elytron and wing, C, D. Female elytron and wing.

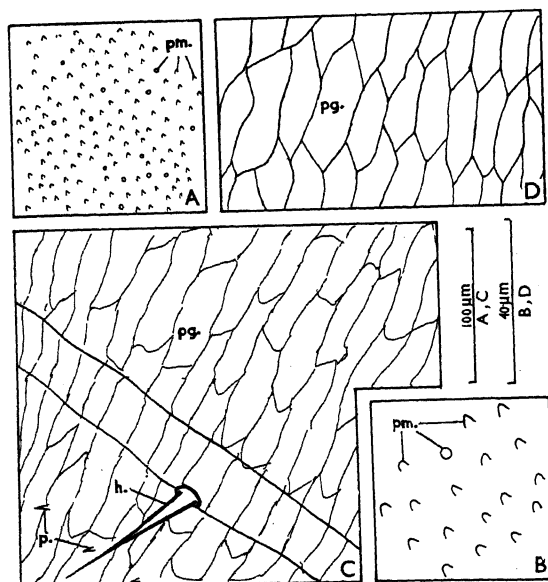


Figure 4. *Supella longipalpa*, male elytron. Views of dorsal surface from regions 1 and 2, to show microsculpturing A. From region No. 1. B. Same, a part more magnified C. From region No. 2. D. same, a part more magnified (h, hairs; p, papillae; pg, polygons; pm, pimplules).

with three types of structures: (i) All over with a pattern of adjacent polygons, ($5\text{--}9\text{ }\mu\text{m} \times 26\text{--}33\text{ }\mu\text{m}$); (ii) a few scattered hairs ($80\text{--}100\text{ }\mu\text{m}$ long) on veins; and (iii) a few minute ($0.5\text{ }\mu\text{m} \times 2\text{ }\mu\text{m}$), thorny papillae scattered on membrane.

3.3a(iii) *region no. 3* (figure 5 A, B): A small region in the middle. Covered with three types of structures: (i) A pattern of adjacent polygons as in region No. 2; present all over except on the veins; (ii) thin, substraight, crescentic or Y-shaped rods (length $10\text{--}15\text{ }\mu\text{m}$, density *ca.* $310/\text{mm}^2$) on the main veins, usually absent on the branches; and (iii) short, thick, nail-like microsetae scattered all over membrane among the polygons; length $7\text{--}14\text{ }\mu\text{m}$, density *ca.* $350/\text{mm}^2$.

3.3a(iv) *region no. 4* (figure 5 C, D): The rest of the surface. Covered all over with a pattern of polygons and microsetae (density *ca.* $300/\text{mm}^2$) as in region No. 2, but without any rods. In addition, a few hairs also present here and there, and an irregular reticulum on the veins.

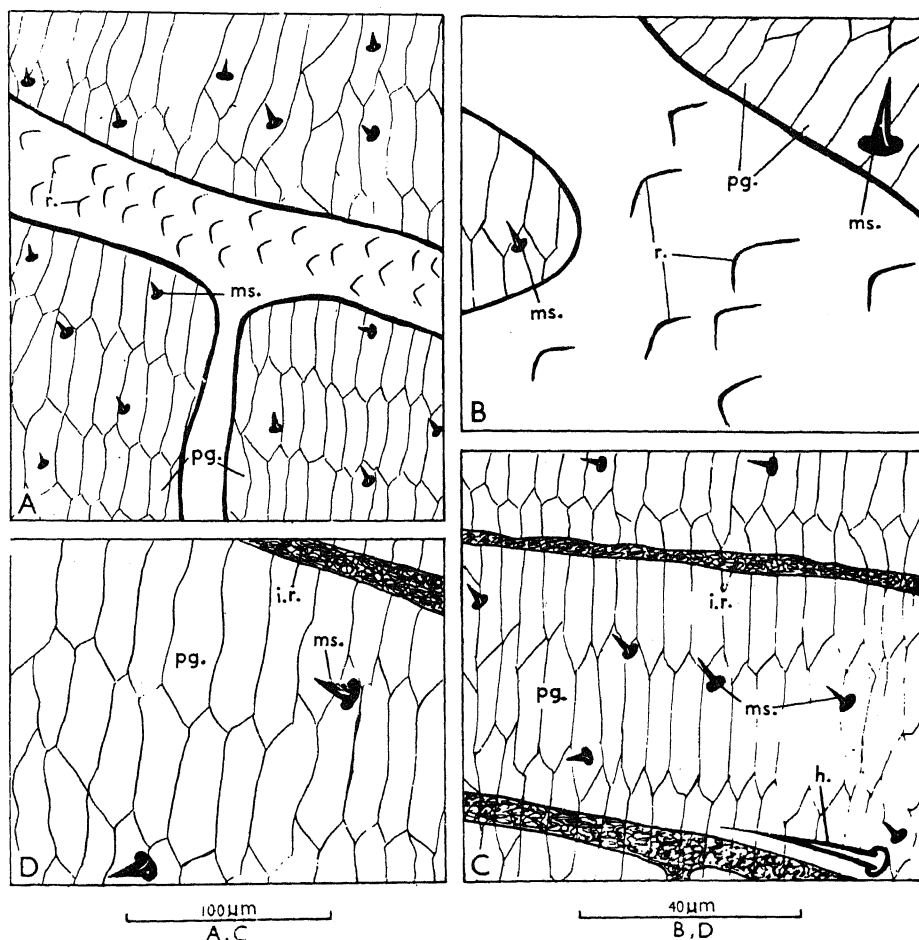


Figure 5. *Supella longipalpa*, male elytron. Views of dorsal surface from regions 3 and 4, to show microsculpturing A. from region No. 3. B. same, a part more magnified C. from region No. 4. D. same, a part more magnified. (h, hairs; i.r., irregular reticulum; ms, microsetae; pg, polygons; r, rods).

3.3b. *Wings*: Length 9.5 mm, maximum width 6.5 mm. Divisible into 3 regions:

3.3b (i) *region no. 1* (figure 6 A): A longish region along the entire anterior margin. Covered with an irregular reticulum of fine lines, and a few hairs and microsetae.

3.3b (ii) *region no. 2* (figure 6 B): A short, triangular region at the distal end. Covered

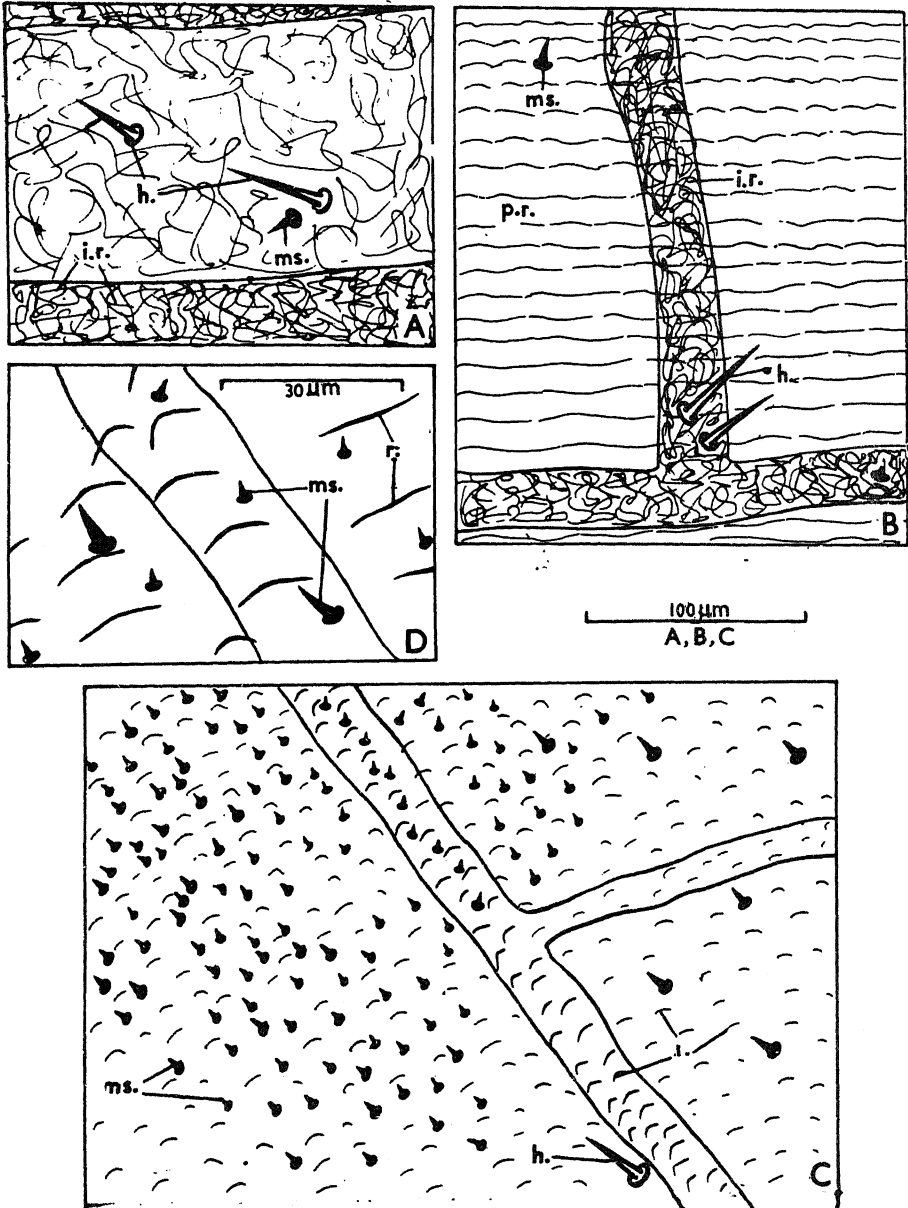


Figure 6. *Supella longipalpa*, male wing. Views of dorsal surface from regions 1-3, to show microsculpturing A. from region No. 1. B. from region No. 2. C. from region No. 3. D. same, a part more magnified. (h, hairs; ir, irregular reticulum; ms, microsetae; p.r, parallel ridges; r, rods).

with a pattern of fine, parallel lines (8-17 μm apart) on membrane, an irregular reticulum on veins and a few microsetae and hairs.

3.3b (iii) *region no. 3* (figure 6 C, D): Therest of the wing forms region 3. Covered with 3 types of structures: (i) Thin rods all over (length 10-15 μm , density *ca.* 320/ mm^2); (ii) microsetae in dense patches (length 3-9 μm , density in patches 370/ mm^2); and (iii) a few scattered hairs..

3.4 *Microsculpturing in females*

3.4a *Elytra*: Length 8 mm, maximum width 3.2 mm. Surface divisible into 4 regions, Nos. 1-4, which are nearly similar in position and extent to those in males. The microsculpturing found here is also similar and need not be described separately.

3.4b. *Wings*: Length 7.5 mm, maximum width 3.3 mm. Surface divisible into 3 regions similar to those in males, and microsculpturing is also similar.

4. Discussion

4.1 *Types of microsculpturing elements*

Eight types (including hairs) of microsculpturing elements have been found on the elytra and wing surfaces of *Supella longipalpa*. Three of these are 'diffuse' and less well-defined than the rest and constitute a pattern of faintly marked polygons, parallel lines and an irregular reticulum. The five remaining ones are well defined, discrete structures, *viz.* pimpules, papillae, rods, microsetae and hairs (setae), ranging in size from 0.5 μm to 100 μm and in density from a few to *ca.* 1000/ mm^2 . The nomenclature adopted for the latter bodies is based on the condition in termites (Isoptera) where these and allied structures have been studied extensively by Roonwal and co-workers (1967-1981; and in press), the identity being based on similarity in size, shape and structure. Whether the similarity also implies homology is difficult to say until more members of the Blattodea have been studied.

Seven types of microsculpturing elements (excluding hairs) have been found in the Isoptera (Roonwal in press a, b). Of these, pimpules, papillae, rods and microsetae also occur in *S. longipalpa*, while the remainder (which are common in the Isoptera), e.g. arrowheads, tubercles and micrasters, are absent, though they may yet be found, along with new types, in other Blattoids. Papillae, which occur universally in the Isoptera (*vide* Roonwal, in press a), are rare in *S. longipalpa*.

Two other 'orthopteroid' orders have been studied. In the small order Zoraptera (Roonwal in press b); a single species, *Zorotypus hubbardi* Caudell, was studied; only hairs are present, fairly thickly, on the wings (length 15-60 μm , density *ca.* 1030/ mm^2).

In the Embioptera (Roonwal and Rathore in press), three types of structures occur on the wings, as follows: Macroscimitars (length 16-150 μm , base swollen); macrotrichia (length 20-130 μm , with a basal platelet; are equivalent to ordinary hairs or setae); and microtrichia (length 8-23 μm). The last named are the most numerous, with density of about 1016-4050/ mm^2 ; they differ from the microsetae of *Supella* and the Isoptera in lacking the basal articulatory platelet.

The 'diffuse' types of microsculpturing, e.g., polygons, parallels and irregular reticulum, described above in *Supella*, have not been reported in the three orders mentioned above.

Table 1. *Supella longipalpa* male. Approximate number of discrete microsculpturing elements on the dorsal surface of each elytron and wing.

Region No. (and its approx. area in mm	Pimpules		Papillae		Rods		Microsetae		Hairs	
	D	N	D	N	D	N	D	N	D	N
<i>A. Elytron</i>										
1 (0.4)	all over 1000 400		—	—	—	—	—	—	—	—
2 (8.5)	—	—	scattered ? A few		—	—	—	—	scattered ? a few	
3 (2.5)	—	—	—	—	1/5th area 310 155		4/5ths area 350 7000		—	—
4 (13.6)	—	—	—	—	—	—	all over 300 4080		scattered ? a few	
Total (25)	—	400	—	A few	—	155	—	11080	—	a few
<i>B. Wing</i>										
1 (7.5)	—	—	—	—	—	—	scattered ? a few		scattered ? a few	
2 (4.5)	—	—	—	—	—	—	scattered ? a few		scattered ? a few	
3 (30)	—	—	—	—	all over 320 9600		dense patches 370 8325		scattered ? a few	
Total (42)	—	—	—	—	—	9600	—	8325	—	a few

D, density (per mm²); N, approximate number of each element; —, absent.

Table 2. Approximate total number of microsculpturing elements in the various regions of a male elytron and wing in *Supella longipalpa*.

Microstructure	Elytron	Wing
Pimpules	400	Nil
Papillae	a few	Nil
Rods	155	9,600
Microsetae	11,080	8,325
Hairs	a few	a few

4.2 Number of microsculpturing elements

It is difficult to count the precise number of microsculpturing elements on elytral or wing surfaces, but a rough approximation can be obtained for a single surface by computing three parameters, viz (i) the area of each region of an elytron or a wing; (ii) the approximate proportion of that area occupied by the elements (distribution is often patchy, e.g. confined to veins or to one corner or margin, etc.); and (iii) the average density of the element in the part where it occurs. Where an element is less confined and is scattered all over, as in the case of hairs, the whole region is considered

as one piece. The total areas of a surface are as follows (for the regions, see table 1):— Males, elytron 25 mm², wing 42 mm²; females, elytron 17 mm² *ca.* 68% of male), wing 16 mm² (*ca.* 38% of male).

The approximate total number of the 5 discrete microsculpturing elements in the various regions on the dorsal surface of a male elytron and wing are given in table 2.

The condition on the ventral side is similar, and the total number of elements on both surfaces may be taken as about double the above figures. Thus, a male elytron carries about 800 pimpules, some 310 rods, 22,160 microsetae and a few scattered papillae and hairs; and a male wing about 19,200 rods, over 16,650 microsetae, a few hairs and no pimpules and papillae. The pimpules are confined to a small region, No. 1, at the base of the elytron; the other structures are more widespread.

The condition in females is similar, but since both elytra and wings are smaller than in males, they carry proportionately fewer elements.

In contrast, the number of elements in the other orders studied is much larger than in *Supella*. In Embioptera the number of microtrichia on a single wing surface goes up to about 34,400, with densities to 4050/mm² (Roonwal and Rathore in press); in the Isoptera it is still higher, up to half a million on a wing surface, with densities as high as *ca.* 13000/mm².

4.3. *Biological and ecological consequences of microsculpturing*

The precise function of microsculpturing is problematic. Females of *S. longipalpa*, being brachypterous, are unable to fly. Males, however, are macropterous and can fly, though weakly. It seems likely that the weak flight may be related, at least to some extent, with microsculpturing, particularly with microsetae of which about 11,080 occur on one surface of an elytron and about 8325 on a wing together with a few of the other types. These structures not only add considerable dead weight to the elytra and wings but also greatly increase surface rugosity. Theoretically, both these features must reduce the aerodynamic efficiency of wings, though not to the same extent as in termites. The reduced flying efficiency may cause a reduction in the ability for dispersal to long distances, and it is thus probable that the widespread occurrence of the species is due largely to external agencies such as commerce rather than to intrinsic effort.

It is also probable that some of the microsculpturing elements, especially microsetae and hairs, may serve as sensillae as in *Blaberus craniifer* (Lefevre 1969).

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On the developmental morphology of the rosette galls of *Acacia leucophloea* Willd., (Mimosaceae) induced by *Thilakothrips bablu* Ramk. (Thysanoptera: Insecta)

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Abstract. *Thilakothrips babuli* induces rosette (artichoke) galls on the axillary buds of *Acacia leucophloea*. As a result of feeding by the building populations of thrips, axillary shoot meristems are destroyed, and primordial palisade tissues of the leaflets and cortical tissues of the shoot axis are transformed into nutritive cells of specialised morphology. Very similar to the galls induced by mites, midges, chalcids, and chloropids, *Thilakothrips*-induced *Acacia* galls also have a characteristic morphology, involving the inhibition of elongation of the shoot axis and crowding of maldeveloped leaflets. While describing the cecidogenesis in relation to the biology of the gall maker, an attempt has been made to discuss the morphological convergence among shoot apex galls.

Keywords. Thrips; *Acacia leucophloea*; *Thilakothrips babuli*; Rosette galls; developmental morphology.

1. Introduction

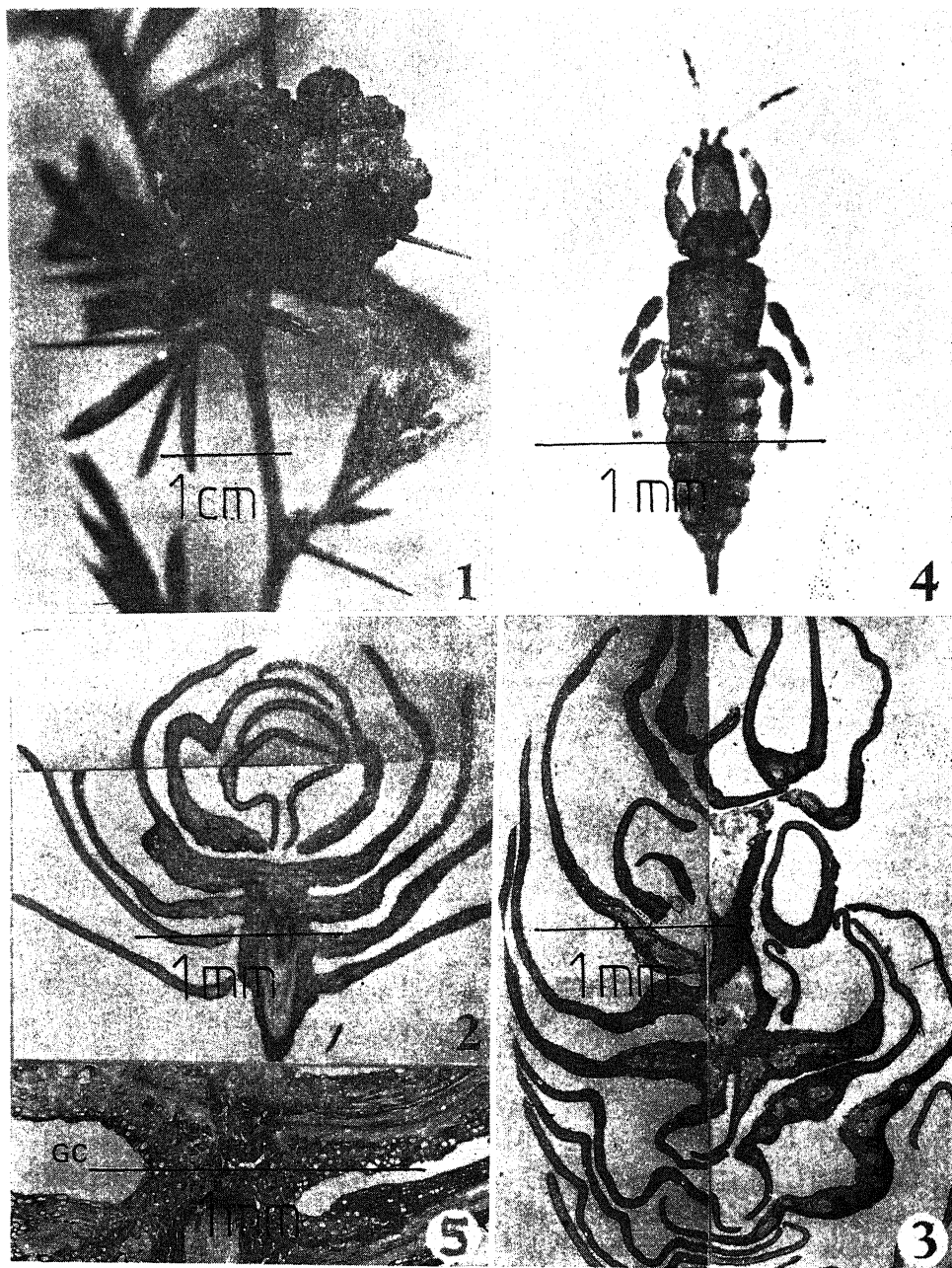
Cecidogenous thrips — *Acacia* association appears striking since more than 30 species of thrips are known to induce galls of diverse morphological patterns on a number of species of *Acacia* in Australia (Mound 1971; Ananthakrishnan 1978). From the Indian subcontinent, Ramakrishna (1928) reported bud galls, known as rosette galls, on *Acacia leucophloea* induced by *Thilakothrips babuli*. *Thilakothrips*-induced *Acacia* galls are unique as bud galls (figure 1) involving rachis, leaflets and portions of the shoot system (figures 2-3) as different from the common leaf galls of Thysanoptera (Ananthakrishnan 1980).

2. Material and methods

Normal and galled shoots of *Acacia leucophloea* were fixed in FAA (formalin; glacial acetic acid; ethanol). Depending upon their relative dimensions and approximate population count supplements, the FAA-fixed galls were graded into various developmental stages and processed through customary methods of dehydration and embedding in wax. Serial sections (6-10 μ m) were stained with safranin-methylene blue and safranin-fast green combinations.

3. Biology of *Thilakothrips babuli*

With the production of new shoots in *Acacia leucophloea* during late April or early May, a few diapausing apterous adults of the preceding generation migrate and

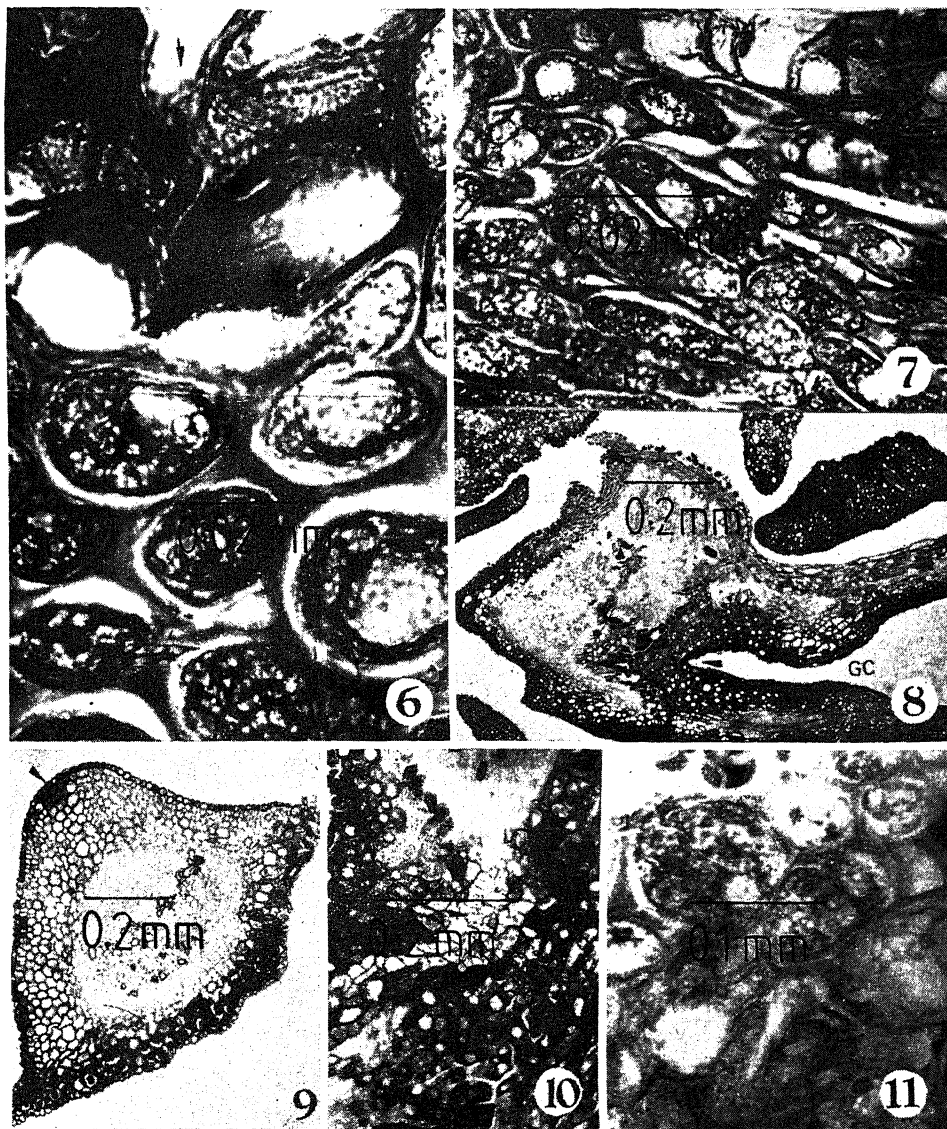


Figures 1-5. 1. Gall of *Acacia leucophloea*. 2. Mature gall in LS. 3. TS mature gall. 4. *Thilakothrips babuli* (♀). 5. LS young gall (GC — Gall chamber).

inhabit the developing buds.. As a result of continuous feeding, the developing buds are galled and gall-development is directly proportional to the building-up of the *Thilakothrips* population. *T. babuli* is highly seasonal and induces galls over a period of 45 days, particularly during the summer months, May and June. Gravid females of *T. babuli* (figure 4) lay 25-34 eggs; the larvae emerge out and become adults in 22 days. The emergence of adults from the 2nd pupae coincides with the formation of young foliar primordia of the host-plant. As with all the gall-formers (Mani 1964) the period of emergence of adults coincides with the optimum range of host development which in turn underlines the basic necessity of the host tissue for the development and oviposition of the gall-maker (Varadarasan and Anantha-krishnan 1981).

4. Developmental morphology of the gall

As a result of the feeding damage (figures 5,11) by a few gravid females migrating from the hibernation sites, gall development commences, ultimately ending in a rosette gall with a highly telescoped main axis and a number of elongated, widened, and thickened (in varying degrees) leaflets. With an increase in number, thrips not only inhabit the apical region of the stem but also spaces between the leaflets. Invariably the apical region of the axillary shoot is completely destroyed by the feeding of thrips. Consequently a characteristic nutritive zone develops both in the superficial layers of the leaflets and in the outer cortical regions of the stem. In leaflets, the feeding is invariably restricted to the adaxial side and this stimulates the palisade cells to divide periclinally, contributing to the thickness of the gall-leaflets (figure 8 — arrow). The epidermal cells and the palisade undergo hyperplasy as well as lateral stretching, facilitating the elongation of gall-leaflet (figures 12,13). The resultant cells constitute the compactly arranged nutritive zone with prominent nuclei and dense cytoplasm (figure 6). With the maturing of the leaflets, concurrent with the development of the gall, the nutritive cells show two different profiles depending on their location. When the cells are closer to the midrib region, they are very large, almost isodiametric, and are characterised by prominent cytoplasm and nuclei (figure 6). On the other hand, the nutritive cells that occur along the extended laminar regions appear greatly stretched along the radial axis and contain some golden-yellow amorphous inclusions (figure 7). In general, the modifications in the morphology of the leaflets are profound, when compared with the normal leaflet (figure 14) the leaflets are elongated in different axes: (i) elongated laterally without any pronounced central thickening (figure 16), (ii) elongated laterally to a moderate degree but thickened to a very great extent in the midrib region, also showing extensive vascular hypertrophy (figure 17), (iii) elongated along only one of the laminar halves to a profound degree (figure 15), (iv) elongated along both the laminar halves displaying a prominent fold (figure 18), and (v) elongated and thickened to a great extent without any folding (figure 19). Whereas these are the general patterns of morphological variations in the leaflets constituting the gall, different grades of thickening have also been observed within a single gall, depending on the position of the leaflet. During gall development the youngest leaflet closer to the axillary shoot apex shows greater thickening as well as an increase in tannin content along

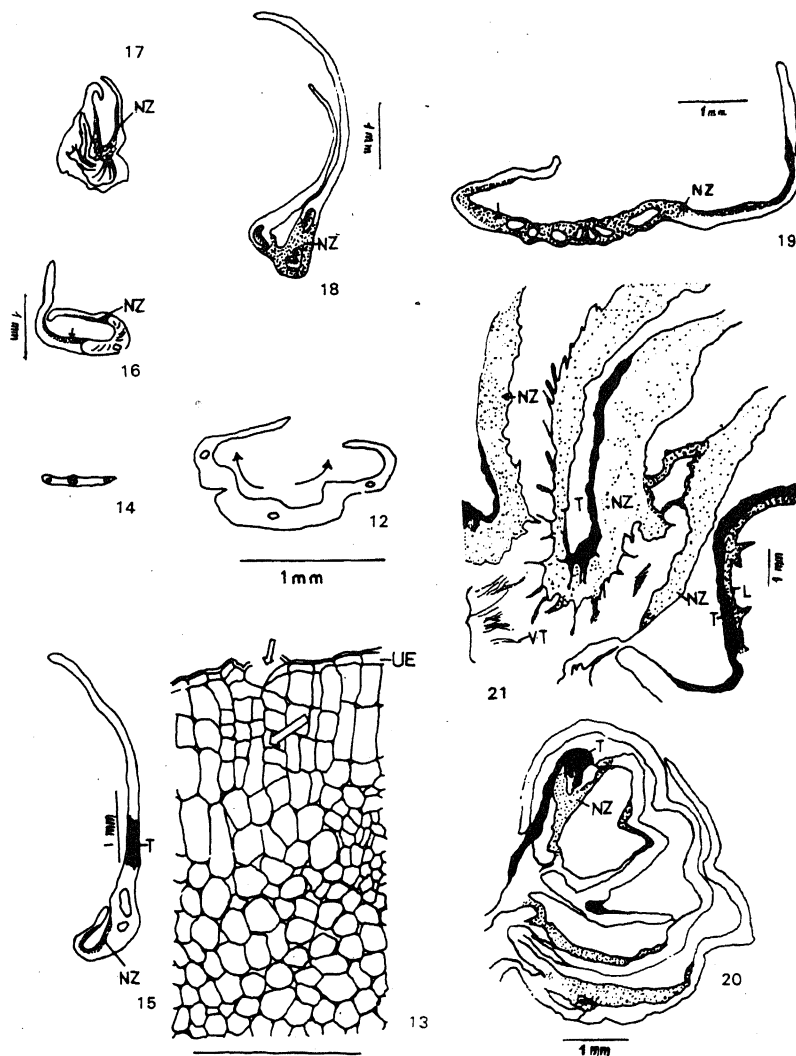


Figures 6-11. 6. TS young gall leaflet (feeding injury - arrow). 7. Nutritive cells — (midrib zone) in a young gall leaflet TS (nutritive cells — laminar zone). 8. TS young gall leaflet (GC — Gall chamber; arrow — nutritive cells). 9. Young gall stem in TS (arrow — feeding site; Darker cells — Tanniniferous cells). 10. Young gall stem in TS (arrow — hyperplasy of cortical cells). 11. TS young stem (nutritive cells).

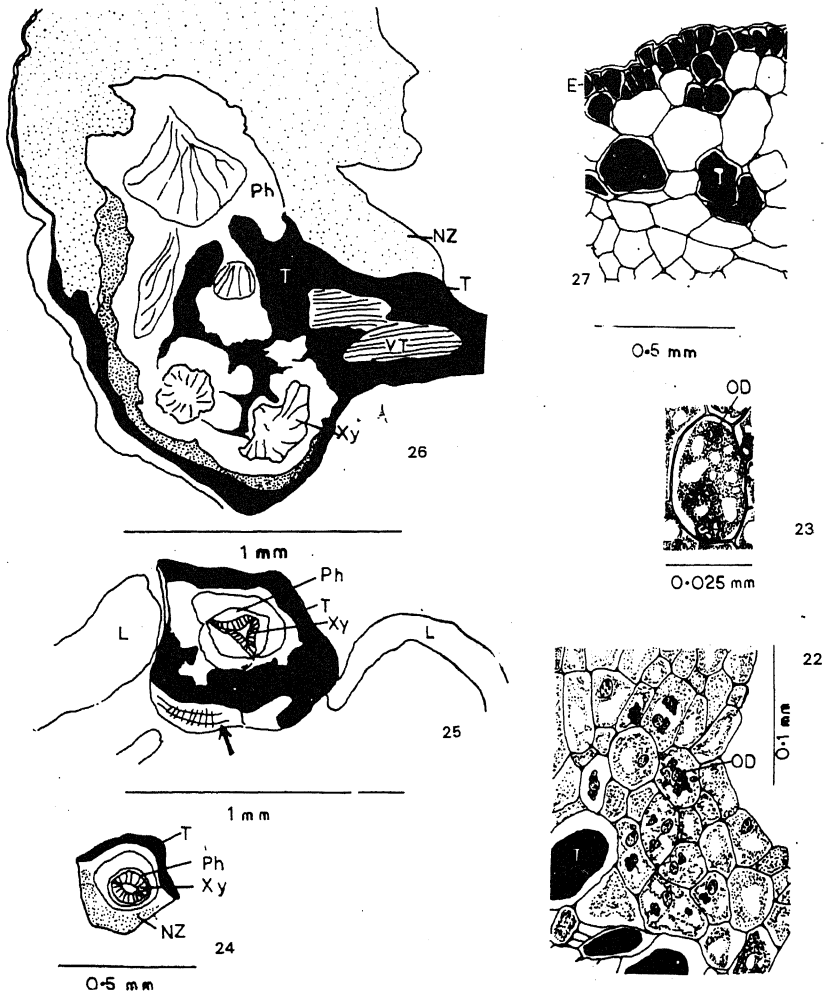
the abaxial epidermis and mesophyll; the thickening gradually gets reduced from the innermost leaflet to the outer one, although elongation is profound in the leaflets away from the shoot apical region (figure 20). Oblique sections of galls show peculiar fusion patterns of leaflets, thus establishing a 'closed' gall system (figure 20), a phenomenon rather unusual among the thrips galls. Leaflets occurring at these sites invariably show development and organization of nutritive

cells of the morphology described earlier. Tannin accumulates in cells in which thrips no longer feed (figure 21).

The infected regions of the main stem show hypertrophy and hyperplasy of the cortical cells (figures 9,11 — arrow). As a result of feeding of thrips, the epidermal and the subjacent layers of the cortex become differentiated into nutritive cells which show large nuclei, dense cytoplasm, and groups of oil droplets (figures 22,23). The cortical cells contribute to the bulk of the galled stem by repeated divisions (figure 10). On account of the proliferation of the interxylary parenchyma (figures 24-26) and phloem, the vascular cylinder becomes disrupted into



Figures 12-21. 12. Young gall leaflet in TS (arrow — inrolling of lamina). 13. Young gall leaflet in TS (arrows — injury and proliferation of the palisade zone). 14. TS mature normal leaflet. 15-19. Gall leaflets in TS (T — tanniniferous zone; VB — Hypertrophied Vascular Bundle). 20. Mature gall in LS (oblique section). 21. Mature gall in LS (accumulation of tannin in old non-functional nutritive zone).



Figures 22-27. 22. Cortical region of gall stem in TS (nutritive cells). 23. A cell enlarged from figure 22 to show abundant cytoplasm and vacuoles. 24. Young gall stem in TS. 25. Mature gall stem TS (arrow — meristematic activity). 26. Old gall stem TS. 27. Cortical region of old gall stem TS (E — Epidermis; T — Tannin).

Abbreviations: OD — oil droplets; NZ — nutritive zone; Ph — phloem; T — tanniferous cells; Xy — xylem; VT — vascular trace; E — epidermis.

numerous isolated strands. The old stem-gall areas show abundant accumulation of tannin which permeate through the central core of the stem (figure 27).

Accumulation of tannin is yet another interesting feature in these galls, which is frequent in normal tissues of *Acacia* spp. as well. Curiously in the gall-tissues, however, tannin accumulates in the regions where thrips have ceased to feed. This aspect is more clearly evident in the stem regions of the gall (figures 26, 27). In the young stems, with the organization of the nutritive zone, the tanniferous zone develops on the opposite side (figure 24). Whether tannin production is inhibited in the nutritive area or occurs in regions where thrips do not feed as a result of the organization of the nutritive zone, is not clear.

Cecidogenesis in *Acacia* galls involves the inhibition of histogenesis, especially in the leaflets and to a moderate extent in the stem regions and the subsequent hypertrophy and hyperplasy. Whereas the shoot apex becomes destroyed, the continued feeding of thrips affects the leaflets occurring beneath; it also inhibits the growth of the shoot axis which naturally results in the telescoped nature of the stem.

5. Discussion

By virtue of being formed on the axillary vegetative buds, *Acacia leucophloea* galls differ significantly from the other *Acacia* galls known to be formed on the phyllodes as large pouches (Mound 1971). By their number, mode of nutrition, and the life-cycle pattern well-synchronised with the developmental phase of the host-plant, *Thilakothrips* affect the axillary shoot meristems, and eventually the normal growth and organogenesis of the host-shoots. This is similar to the cecidogenetic influence of mites such as *Cecidophyopsis psilapsis* (Nal.) Keiffer, *C. ribis* Westw., *Phytophus avellanae* (Nal.), and *Aceria thomasi* (Nal.) inducing artichoke galls on the shoot apices of *Taxus baccata* L., *Ribes nigrum* L., *Corylus avellana* L., and *Thymus serpyllus* respectively (Dréger-Jauffret 1977). Leaflets of *Acacia* galls, examined here, show different degrees of maldevelopment possibly because the affected organ is a bud with foliar primordia in different stages of development. Besides the tendency to form 'closed' gall-systems, patterns of modifications in the leaflets with a number of intergrades from simple crinkles to folds and rolls, are indeed impressive. Variations in the leaflets of the gall-shoot pertain not only to size, but also to morphological elaboration in terms of the regenerative capacity and the inherent growth potential. This is well substantiated by the more-active growth centres in the foliar areas than in the axis areas, the latter showing inhibition of elongation.

The general morphogenetic pattern in these thrips-induced *Acacia* galls conforms to that of the bud-galls induced not only by mites (Westphal 1977; Dreger-Jauffret 1977), but by cecidomyiids (Jauffret *et al* 1970), cynipids, chloropids, and chalcids (Dreger-Jauffret 1977) as well, showing identical telescoping effect of the shoot axis. This is probably because of the destruction of the shoot apical meristem during early or late stage of gall-development. Besides, more than one gall maker is involved in cecidogenesis, requiring the organization of specialised nutritive tissue. Probably, a functional compromise is achieved among the destruction of the apical meristem, the organization of nutritive cells all over the gall region, and the normal organogenetic effort of the host in order to contribute to the rosette form of the gall.

Curiously, the galls formed on the shoot apices of diverse host plants display almost identical gall-forms, in spite of being induced by cecidozoa of different orders, indicating a morphological convergence. This is specially significant since galls formed on other plant organs such as leaf, stem, etc., by cecidozoa of the same order are generally significantly different morphologically (Westphal 1977; Meyer 1969).

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Biometric comparison of *Nemipterus japonicus* (Bloch) populations along the east coast of India

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Abstract. Morphometric comparison of the samples of *Nemipterus japonicus* populations from four different localities on the east coast of India is made. Analysis of covariance of morphometric characters reveals significance at 1% level for three characters. Character by character comparison of the means of locality-samples shows that there is clinal gradation in maxillary length (south to north) and pectoral length (north to south). The distance function (D)² analysis shows that Kakinada is the farthest removed from other localities, and Visakhapatnam and Madras samples are nearer to each other. Paradeep sample occupied an intermediate position among the locality-samples. It is concluded that all *Nemipterus japonicus* populations along the east coast of India are from a single genetic population, with restricted and localized movements confined to the areas where they are caught. The observed distinctness of Kakinada sample appears to be due to phenotypic variation under the influence of low salinity.

Keywords. Distance function; analysis of covariance; biometric comparison; locality-samples; *Nemipterus japonicus*.

1. Introduction

The Japanese threadfin bream, *Nemipterus japonicus* (Bloch), the most common of the eight species of the genus recorded from Indian waters, forms an important fishery among the demersal catches landed along the Indian coast. The species contributed to about 13% of the total trawl catches landed at Visakhapatnam Fishing Harbour during July 1977-June 1980 (Rao 1981).

The biometric features of *N. japonicus* caught off Porto Novo on the east coast of India were studied by Selva Kumar (1971) and Nammalwar (1973). Hsu and Liu (1977) compared morphometric characters of samples of *N. virgatus* obtained from the waters off Northern Sunda, East China Sea, Taiwan Straits, South China Sea and the Gulf of Tonkin. No comparison of *N. japonicus* populations from different localities has been made hitherto. The present comparison of *N. japonicus* was made with a view to judge the homogeneity or otherwise of the populations based on the samples obtained from Paradeep, Visakhapatnam, Kakinada and Madras on the east coast of India (figure 1).

2. Material and methods

All the samples were drawn randomly from the landings of small mechanised fishing boats equipped with trawl nets (cod-end mesh size ~ 22 mm). The samples were preserved in 5% neutral formalin for about a fortnight before measurements were taken. Body dimensions of 40 specimens each from four localities were taken for statistical analysis.

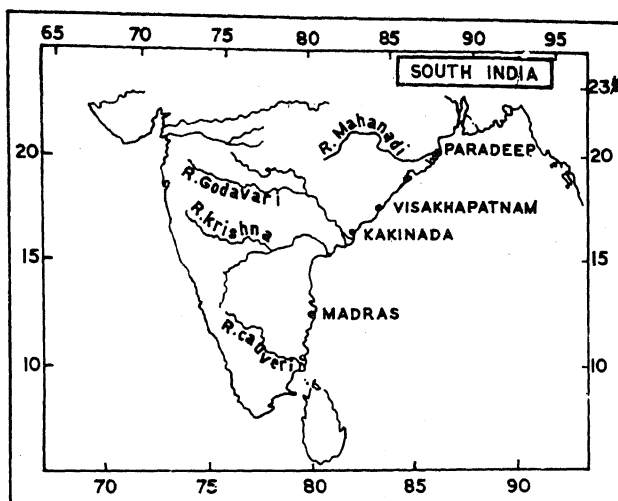


Figure 1. Sampling localities.

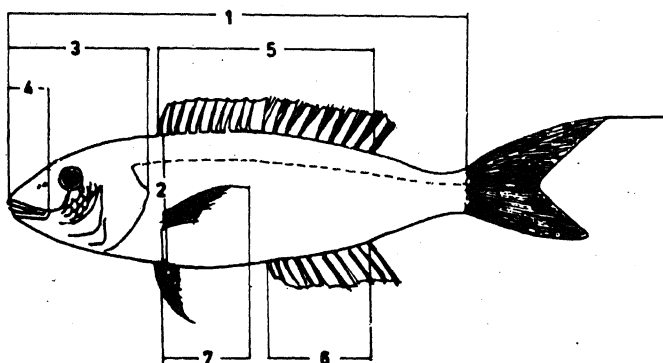


Figure 2. Schematic diagram of *Nemipterus japonicus* showing morphometric measurements. 1. Standard length 2. Height of the body 3. Head length 4. Maxilla length 5. Length of dorsal base 6. Length of anal base 7. Pectoral length.

The morphometric characters selected were (i) standard length (ii) height of the body (iii) head length (iv) maxilla length (v) length of dorsal base (vi) length of anal base and (vii) pectoral length as shown in figure 2. Standard length was taken as the independent variable (X_0) and all other characters as dependent variables (X_1 X_8). Height of the body, head length and pectoral length were measured following Royce (1964). The measurements made to the nearest millimeter using a pair of fine dividers were taken on the left side of the body along a straight line. The computations were made using an IBM 1130 electronic computer.

3. Physical features of localities

The localities from where samples were collected are shown in figure 1.

Paradeep: ($23^{\circ} 30' N$ $86^{\circ} 20' E$) situated at the confluence point of the river Mahanadi. The discharge of the river water has its influence on the salinity of the seawater.

Visakhapatnam: (17° 42' N 83° 20' E) The coast is shingly in nature, interspersed by wide stretches of sandy areas. There is no large scale freshwater influx into the sea.

Kakinada: (16° 57' N 82° 15' E) The northern-most distributary of the river Godavari enters into the region bringing about great changes in the salinity of seawater over a wide area.

Madras : (13°04' N 80°17'E) The locality is an open coastline with sandy beach. There are no large rivers entering the sea.

4. Computations

4.1 Analysis of covariance

The regression coefficients (*b* values) of the body characters in relation to standard length, of the samples collected from different localities, are shown in table 1. The *F*-values along with the error mean square values are also given in table 1.

4.2 Character by character comparison of the means of the samples

The graphical method of Hubbs and Hubbs (1953) for character by character comparison on the basis of linear regression analysis is carried out in the manner modified and adopted by Royce (1964) and Rao (1975). The overall mean of standard length in the present study is 11.45 cm (table 3), which is used for the calculation of one standard deviation ($S_{y,x}$)* and two standard errors of the mean ($2S_{y,x}$)**. The results are arranged in a geographical sequence (figure 3).

The extent of closeness of the samples to one another and to Visakhapatnam sample was further tested on the basis of distance function (D^2) which takes into account all the characters simultaneously.

4.3 Calculation of D^2

The computational procedure for the calculation of distance function (D^2) is according to the method given by Rao (1952 Procedure B1 in Appendix B, Chapter 8), which was suitably modified later by Rao (1975) taking into account the regression statistics.

* One standard deviation is calculated as

$$S_{y,x} = (\sum y^2 - (\sum xy)^2 / \sum x^2 / n - 2)^{1/2}$$

** Two standard errors of the mean are calculated as

$$2S_{y,x} = 2S_{y,x} (1/n + x^2 / \sum x^2)^{1/2}$$

where $\sum x^2$, $\sum y^2$ and $\sum xy$ are corrected sums of squares and products (Appendix I); *n*=number of fish in each sample; x^2 is the square of the difference between the sample mean of standard length and the overall mean of standard length.

Table 1. Regression coefficients corresponding to different body dimensions (b_1 b_6) regressing on standard length (X_0)

Locality	Sample Size	Regression coefficients					
		b_1	b_2	b_3	b_4	b_5	b_6
Paradeep	40	0.3393	0.3169	0.1196	0.5048	0.1984	0.3353
Visakhapatnam	40	0.3206	0.2860	0.1008	0.4952	0.1989	0.3057
Kakinada	40	0.3361	0.2835	0.1048	0.5198	0.1791	0.2731
Madras	40	0.3636	0.3305	0.1195	0.4903	0.1712	0.3518
Overall	160	0.3428	0.3079	0.1137	0.5047	0.1862	0.3188
F-values resulting from comparison of regression coefficients †							
Error mean square		2.9214**	11.3638*	5.8119*	1.3294**	3.7081**	15.6257**
		0.0108	0.0061	0.0019	0.0177	0.0071	0.0120

X_1 = Height of the body; X_2 = Head length; X_3 = Maxilla length; X_4 = Length of dorsal base;
 X_5 = Length of anal base; X_6 = Pectoral length

† degrees of freedom (3,152); *Significant at 1% level; **Not significant

Table 2. Mean values of the different body dimensions for each locality.

	Sample size	Size range (cm SL)	Mean $\pm$ SD							
			$\bar{X}_0 \pm SD$	$\bar{X}_1 \pm SD$	$\bar{X}_2 \pm SD$	$\bar{X}_3 \pm SD$	$\bar{X}_4 \pm SD$	$\bar{X}_5 \pm SD$	$\bar{X}_6 \pm SD$	
Paradeep	40	9.2-18.7	11.32 $\pm$ 2.35	3.57 $\pm$ 0.80	3.35 $\pm$ 0.75	1.21 $\pm$ 0.28	5.73 $\pm$ 1.19	2.01 $\pm$ 0.47	3.19 $\pm$ 0.79	
Visakhapatnam	40	9.7-14.6	11.80 $\pm$ 1.11	3.78 $\pm$ 0.36	3.45 $\pm$ 0.33	1.24 $\pm$ 0.12	5.87 $\pm$ 0.58	2.09 $\pm$ 0.24	3.23 $\pm$ 0.35	
Kakinada	40	7.4-20.4	10.66 $\pm$ 2.06	3.40 $\pm$ 0.70	3.14 $\pm$ 0.59	1.14 $\pm$ 0.22	5.40 $\pm$ 1.07	1.92 $\pm$ 0.38	2.97 $\pm$ 0.57	
Madras	40	9.1-17.1	12.03 $\pm$ 1.89	3.81 $\pm$ 0.70	3.59 $\pm$ 0.63	1.30 $\pm$ 0.23	6.01 $\pm$ 0.94	2.13 $\pm$ 0.34	3.55 $\pm$ 0.68	
Overall	160	7.4-20.4	11.45 $\pm$ 1.96	3.64 $\pm$ 0.73	3.38 $\pm$ 0.61	1.22 $\pm$ 0.23	5.75 $\pm$ 0.99	2.04 $\pm$ 0.37	3.23 $\pm$ 0.65	

Table 3. Pooled variance-covariance matrix.

	X_0	X_1	X_2	X_3	X_4	X_5	X_6
X_0	3.6489	1.2509	0.4150	1.1635	0.6326	0.5679	0.1337
X_1		1.1238	1.8419	0.4400	0.2316	0.2088	0.9475
X_2			0.6795	0.3904	0.4035	0.3641	0.3462
X_3				0.1437	0.3625	0.0493	0.5880
X_4					0.1295	0.2099	0.1340
X_5						0.0771	0.2168
X_6							0.3863

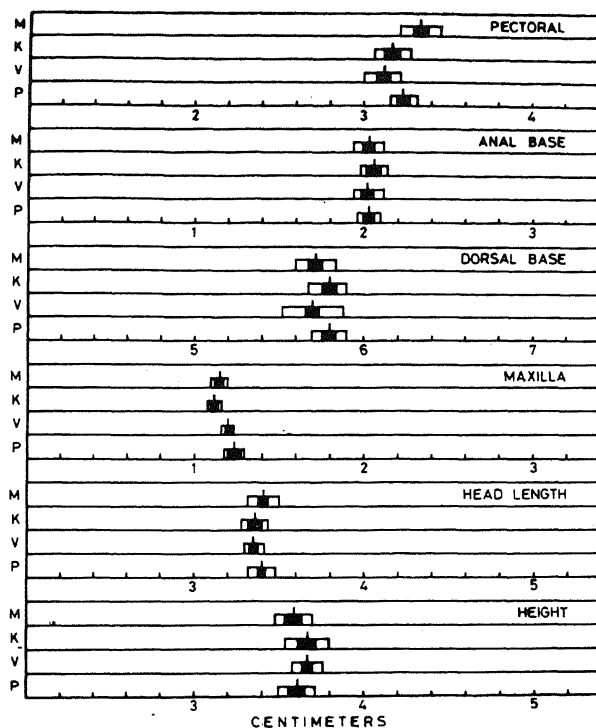


Figure 3. Mean lengths of body dimensions of *Nemipterus japonicus*. The centre line indicates the mean, the hollow bar $\pm$ one standard deviation and the solid bar $\pm$ two standard errors of the mean as computed from regression statistics. P—Paradeep; V=Visakhapatnam; K—Kakinada; M—Madras.

Table 4. b_{ij} values.

	X_0	X_1	X_2	X_3	X_4	X_5	X_6
X_0	3.6489	1.2509	0.4150	1.1635	0.6326	0.5679	0.1337
X_1		0.6950	1.6996	0.0411	0.0147	0.0141	0.9017
X_2			-3.5243	0.1575	0.2955	0.2650	-1.8741
X_3				-0.2227	0.1641	-0.1208	0.4082
X_4					0.1652	0.0443	0.2354
X_5						0.0620	-0.2479
X_6							-0.3692

Table 5. Transformed normalized mean values (y_i s).

Locality	X_0	X_1	X_2	X_3	X_4	X_5	X_6
Paradeep	-0.0697	-0.0352	0.0290	-0.0660	-0.0616	0.0460	0.0897
Visakhapatnam	0.1803	0.0263	-0.0135	-0.2026	0.3227	-0.3356	-0.6739
Kakinada	-0.4126	0.0392	-0.1253	0.3746	-0.8016	0.8076	0.8860
Madras	0.3020	-0.0303	0.1098	0.2379	0.5406	-0.5181	-0.3018

Table 6. Values of D^2 arranged in increasing order of magnitude. F values with 6,73 degrees of freedom are given in parenthesis.

Paradeep (1)	Visakhapatnam (2)	Kakinada (3)	Madras (4)
(4) 0.9329 (2.91)	(4) 0.2389	(1) 1.8863 (5.88)*	(2) 0.2389 (0.75)
(2) 0.9540 (2.98)	(1) 0.9540	(2) 5.3499 (16.69)*	(1) 0.9329
(3) 1.8863	(3) 5.3499	(4) 5.4050 (16.86)*	(3) 5.4050

*Significant at 1% probability level.

Using the notation of Rao (1952) and Rao (1975), the normalized mean values $x_1, \dots, x_p$ were transformed to values of $y_1, \dots, y_p$, which have unit standard deviation. The values of y were obtained through the formulae given below† following the procedure given by Rao (1975). The final D^2 values corresponding to any two groups is the sum of squares of differences in transformed normalized mean values of all characters (excluding X_0). The results are presented in tables 3 to 6.

5. Results

5.1 Analysis of covariance

Though the regression coefficients for the different samples are numerically close to one another the F -values comparing the coefficients are significant at 1% level for three characters, namely head length, maxilla length and pectoral length (table 1).

5.2 Character by character comparison

The overlap of the solid bars (two standard errors) indicates the closeness of different locality-samples with regard to each character (figure 3). The overlapping was very

†The values of y were obtained through the formulae

$$y_i = x_i - \sum_{j=1}^{i-1} (b_{ij} / (b_{jj})^{1/2}) y_j / (b_{ii})^{1/2} \text{ for } i = 0, 1, \dots, 6$$

where $b_{11} = \lambda_{11}$

$$b_{ij} = \lambda_{ij} - \sum_{t=1}^{j-1} b_{jt} b_{it} / b_{tt} \quad \text{when } i > j$$

$$\text{and } b_{jj} = \lambda_{jj} - \sum_{t=1}^{j-1} b_{jt}^2 / b_{tt} \text{ and } b_{ij} = b_{ji}$$

The formulae given above are obtained on substituting the values of a_{ij} (Rao 1952).

Appendix 1. Corrected sums of squares and products of different body dimensions for four localities

	X ₀	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆
Paradeep (n = 40)							
X ₀	215.59	73.17	68.34	25.79	108.83	42.78	72.29
X ₁		25.25	23.37	8.83	37.04	14.49	24.63
X ₂			21.88	8.24	34.61	13.59	22.99
X ₃				3.17	13.10	5.13	8.70
X ₄					55.27	21.71	36.59
X ₅						8.66	14.33
X ₆							24.58
Visakhapatnam (n = 40)							
X ₀	48.71	15.62	13.94	4.91	24.13	9.69	14.89
X ₁		5.28	4.57	1.59	7.95	3.14	4.92
X ₂			4.14	1.43	7.08	2.81	4.36
X ₃				0.56	2.48	0.95	1.50
X ₄					13.22	4.88	7.52
X ₅						2.30	3.11
X ₆							5.02
Kakinada (n = 40)							
X ₀	164.93	55.44	46.76	17.30	85.73	29.54	45.06
X ₁		19.14	15.94	5.85	28.83	9.89	15.31
X ₂			13.53	4.97	24.30	8.32	12.95
X ₃				1.88	9.01	3.09	4.78
X ₄					45.11	15.46	23.44
X ₅						5.54	8.02
X ₆							12.76
Madras (n = 40)							
X ₀	139.99	50.91	46.27	16.73	68.64	23.98	49.26
X ₁		18.97	17.02	6.15	24.86	8.61	18.09
X ₂			15.59	5.56	22.61	7.85	16.49
X ₃				2.08	8.16	2.86	5.88
X ₄					34.21	11.96	24.17
X ₅						4.41	8.35
X ₆							17.90

high with regard to head length and anal base at all the localities. The overlap of the solid bars is 100% for height of the body between distantly situated Paradeep and Madras samples as well as between samples from Visakhapatnam and Kakinada which are geographically close to each other. With regard to maxilla, apart from Madras, there is an increasing trend from south to north (Kakinada to Paradeep). Similarly for pectoral length also, barring the Paradeep sample, there is an increasing trend from north to south (Visakhapatnam to Madras). In dorsal base, Paradeep and Kakinada samples showed extreme overlap and there is considerable overlap between Visakhapatnam and Madras also.

Character by character comparison of the samples of different localities may be summarized as follows: (i) greater heights of Visakhapatnam and Kakinada samples compared to other samples (ii) longer heads of Paradeep and Madras samples compared to other samples (iii) longer maxillae of Paradeep and Visakhapatnam samples (iv) longer dorsal bases of Paradeep and Kakinada samples (v) longer pectoral of Madras sample.

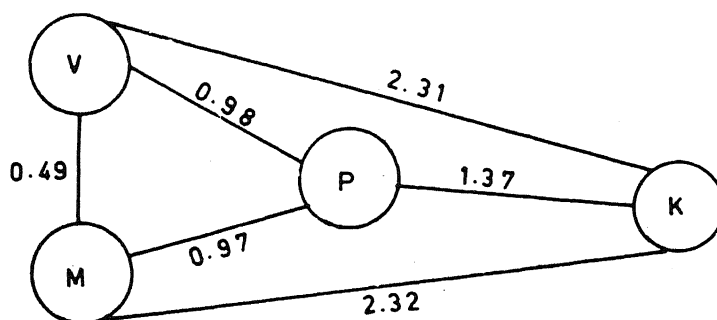


Figure 4. Clusters of locality groups of *Nemipterus japonicus* according to standard lengths and their relationships. P—Paradeep; V—Visakhapatnam; K—Kakinada; M—Madras.

But for the aberrant deviations from a trend line in either Kakinada sample or Paradeep sample or both, there is clinal gradation which is most clearly seen in pectoral and maxillary lengths. The Kakinada and Paradeep samples showed a great degree of overlap of anal base, dorsal base and head length, but the two samples were also found to show no overlap with regard to maxillary length. This shows that the physico-chemical similarity due to freshwater discharge into the sea at Kakinada and Paradeep is probably responsible for the high degree of overlap with regard to anal base and dorsal base while the maxillary length which showed clinal gradation is not influenced in the same manner.

5.3 D^2 analysis

The D^2 values were arranged in an increasing order corresponding to each locality (table 6). Figure 4 shows that Visakhapatnam and Madras samples are very close to each other and are probably from the same population. Kakinada sample is the farthest removed from the other three locality-samples. Kakinada sample is almost at equal distance from Visakhapatnam (5.35) and Madras (5.4) samples, but is somewhat nearer to Paradeep sample (1.89). Paradeep sample, which is between the Visakhapatnam-Madras complex on the one hand and Kakinada sample on the other, is a little nearer the former (0.95 with Visakhapatnam and 0.93 with Madras) than the latter (1.89). The relationships thus show that Kakinada sample is the farthest removed and remains distinct from the Paradeep sample also, which occupies an intermediate position between the Visakhapatnam-Madras complex and Kakinada.

6. Discussion

Since all the samples from different localities were collected within an interval of 50 days (January and February 1980), some amount of homogeneity can be assumed at each locality as the specimens belong to the same size group (table 2). Kakinada is geographically near Visakhapatnam (145 km). Paradeep and Madras are respectively 455 and 630 km away from Visakhapatnam. So, there is greater scope of intermingling of populations between Visakhapatnam and Kakinada than between Visakhapatnam and Paradeep or between Visakhapatnam and Madras.

Analysis of covariance gave values significant at 1% probability level for head length, maxilla and pectoral length. Character-by-character comparison revealed that the observed differences between samples from different localities were not so high as

to consider that independent populations exist at the localities. However, taking the overlapping of the two standard errors of the mean as the basis for comparison, a certain degree of similarity is observed between the Visakhapatnam and Madras samples with regard to head length, dorsal and anal bases. Visakhapatnam sample also showed some proximity in head length and anal base with Paradeep and Kakinada samples. On the other hand, Paradeep and Kakinada samples showed some closeness in head length, dorsal and anal bases and to a little extent in pectoral also.

Using all the characters simultaneously, as in D^2 statistic, the degree of closeness or distance of the samples from one another is judged. The statistic (D^2) is significant when Kakinada sample is compared with samples from other localities (table 6). In spite of the greater scope for intermingling of populations off Visakhapatnam and Kakinada, which are geographically closer, the D^2 value of Kakinada with Visakhapatnam is comparatively high (5.35). The D^2 Value of Kakinada with Madras, which are geographically distant, is also of the same magnitude (5.4). Instances of geographically closer localities having higher D^2 values were encountered by Pillay *et al* (1963), Royce (1964) and Rao (1975).

Since geographically close samples are as distinct with regard to D^2 statistic as geographically well-separated samples, it may be inferred that the observed morphological differences are not due to latitudinal differences. The closeness of the Madras and Visakhapatnam samples may be attributed to the fact that these were caught from areas without freshwater influx. Compared to these two places, Kakinada and Paradeep are different in being situated at the mouths of perennial rivers and out of these two, Kakinada receives greater inflow of freshwater from the adjoining river systems (Rama Sarma 1965). Lower salinities at Kakinada and Paradeep seem to affect the morphometrics of *N. japonicus*. The lower the salinity, as at Kakinada, the greater the influence. This phenomenon was also observed by Rao (1975) in *Scomberomorus guttatus*. Hence the intermediateness of Paradeep sample between Kakinada and Visakhapatnam-Madras complex, which strongly indicates that the observed morphological differences are of phenotypic origin rather than genotypic. But for the phenotypic differences, all the *Nemipterus japonicus* populations on the east coast of India appear to belong to a single genetic population, with restricted and localized movements, showing morphological differences under the influence of local physico-chemical conditions.

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Prediction of bioenergetics components of lepidopterous larva

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Abstract. Using the bioenergetics data for the final instar *Achaea janata* larva exposed to a wide range of ration levels and temperatures, a linear regression model to predict the bioenergetics components of lepidopterous larva has been developed. From the dry weight of faeces egested (F) it is possible to estimate the consumption (C), production (P) and metabolism (R). Validity of the proposed model has been tested using the data reported in literature.

Keywords. Lepidoptera; bioenergetics components; regression model; *Achaea janata*.

1. Introduction

Reviewing the nutritional ecology of immature insects, Scriber and Slansky (1981) emphasised the significance of quantitative data on food consumption and utilisation in insects for a better understanding of their ecology. Experiments on intake of natural diets usually involve gravimetric measurements (Waldbauer 1968), and are probably among the most tedious in the repertory of experimental entomologists, even with the advent of modern electronic microbalances. Apparently, the complexity of the data collecting process accounts for the relatively scanty work in this area (Klein and Kogan 1974). Literature survey reveals stray attempts to devise indirect methods to collect data on food consumption (Bhattacharya and Waldbauer 1969; Holter 1973; Mathavan and Pandian 1974). However, a comprehensive model that can be followed to determine all the bioenergetics components — consumption (C), production (P) and metabolism (R) is yet to emerge.

2. Material and methods

In lepidoptera, over 80% of the total food consumption for the entire larval period occurs during the final instar (Waldbauer 1968; Muthukrishnan and Pandian 1983a). Therefore, bioenergetics data reported earlier (Muthukrishnan and Pandian 1983b) for the final instar *Achaea janata* L. (Noctuidae; Lepidoptera) reared on a wide range of rations (50 to 500 mg *Ricinus communis* leaf per larva per day) at 22, 27, 32 and 35°C were chosen to develop a linear regression model so as to predict the bioenergetics components of lepidopterous larva. The ease and accuracy with which faeces (F) egested by the larvae can be estimated have prompted several workers to choose either components of F (sugar: Krishna and Saxena 1962; uric acid: Bhattacharya and Waldbauer 1969, 1970) or whole F

(Mukerji and Guppy 1973; Mathavan and Pandian 1974) as an index of C . On the same ground, whole F has been chosen to predict C , P and R in the present study.

Use of F in terms of dry weight for obtaining C , P and R in terms of energy may find wide application in studies on energy flow through consumer populations, as it circumvents the estimation of calorific content. But, before choosing to use mass value of F , it is essential that extrinsic factors like temperature and availability of food do not alter the calorific content of F . Analysis of variance of calorific values of faeces egested by final instar *A. janata* exposed to different rations and temperatures revealed that neither of them significantly varied the calorific values of F (table 1). Therefore, mass values of F have been used for predicting C , P and R in terms of energy (Joules).

Data on rates of defaecation (F), consumption (C), production (P) and metabolism (R) of final instar *A. janata* as functions of ration and temperature are presented in table 2. Statistical analysis of the data revealed that F had significant positive correlations with C , P as well as R . Therefore, separate linear regression equations were calculated for the relation between F and C , F and P and F and R . P and R of *A. janata* larva receiving lowest rations at the tested temperatures were low and affected the linear relation of P and R with F ; hence these values were not considered for the computation of equations to predict P and R . Using the regression equations, C , P and R in respect of known F values were predicted. Goodness of fit of the predicted values with the observed data was tested by chi-square method (Zar 1974).

Table 1. Calorific content (J/mg dry matter) of faeces egested by final instar *Achaea janata* larva fed on different rations of *Ricinus communis* leaf (mg/larva/day) at the tested temperatures.

Ration	22°C	27°C	32°C	35°C
50	16.5 ± 0.7	17.4 ± 0.9	*	*
100	17.2 ± 1.1	17.0 ± 0.3	17.7 ± 0.9	16.9 ± 0.7
200	16.8 ± 0.5	17.1 ± 0.6	16.6 ± 0.9	17.5 ± 0.4
300	17.0 ± 0.4	17.8 ± 0.8	17.0 ± 0.9	17.1 ± 0.5
400	17.6 ± 0.7	17.2 ± 0.5	16.9 ± 0.4	17.0 ± 0.7
500	*	*	16.9 ± 0.3	16.9 ± 0.5
<i>Ad libitum</i>	17.9 ± 0.6	17.8 ± 0.9	17.1 ± 0.4	17.4 ± 0.5

Analysis of variance

Source	SS	Df	MS	F-ratio	P**
Total	37.245	76	—	—	—
Between Rations	2.789	4	0.697	1.508	0.1
Between Temperatures	1.514	3	0.505	1.093	0.1
Interaction	6.603	12	0.550	1.190	0.1
Residual	26.339	57	0.462	—	—

Each value ($\bar{X} \pm SD$) represents the mean of minimum 3 estimations.

*For want of comparable data, values at 50 and 500 mg ration levels were not considered for the analysis of variance. ** $P > 0.05$ is not statistically significant.

Table 2. Rates of defaecation (*F*) (mg/g larva/day), consumption (*C*), production (*P*) and metabolism (*R*) (kJ/g larva/day) of *A. janata* as functions of ration level (mg leaf/larva/day) and temperature.

Ration	<i>F</i>	<i>C</i>	<i>P</i>	<i>R</i>
22°C				
50	25.95	1.288 ± 0.121	0.116 ± 0.020	0.745 ± 0.089
100	41.2	1.868 ± 0.203	0.414 ± 0.089	0.744 ± 0.100
200	69.4	2.980 ± 0.473	0.888 ± 0.111	0.924 ± 0.103
300	88.7	3.658 ± 0.473	1.089 ± 0.099	1.061 ± 0.107
400	105.7	4.764 ± 0.731	1.374 ± 0.057	1.528 ± 0.169
<i>Ad libitum</i>	123.8	5.249 ± 0.245	1.477 ± 0.084	1.550 ± 0.298
27°C				
50	38.3	1.693 ± 0.018	0.069 ± 0.015	0.955 ± 0.022
100	61.4	2.498 ± 0.221	0.419 ± 0.074	1.032 ± 0.082
200	98.9	3.980 ± 0.183	1.011 ± 0.028	1.274 ± 0.132
300	117.1	4.951 ± 0.393	1.354 ± 0.185	1.518 ± 0.105
400	132.8	5.524 ± 0.401	1.448 ± 0.223	1.793 ± 0.257
<i>Ad libitum</i>	170.4	7.176 ± 0.378	1.95 ± 0.120	2.152 ± 0.172
32°C				
100	64.4	2.639 ± 0.124	0.372 ± 0.021	1.130 ± 0.145
200	92.7	3.939 ± 0.231	0.913 ± 0.062	1.483 ± 0.127
300	116.6	5.042 ± 0.167	1.227 ± 0.068	1.829 ± 0.152
400	129.3	5.946 ± 0.033	1.539 ± 0.044	2.220 ± 0.093
500	147.8	6.767 ± 0.537	1.890 ± 0.181	2.373 ± 0.283
<i>Ad libitum</i>	161.6	7.405 ± 0.791	2.041 ± 0.213	2.593 ± 0.225
35°C				
100	68.3	2.766 ± 0.108	0.021 ± 0.005	1.588 ± 0.079
200	97.1	4.260 ± 0.358	0.559 ± 0.094	2.005 ± 0.133
300	117.5	5.149 ± 0.436	1.134 ± 0.200	2.037 ± 0.177
400	142.0	6.404 ± 0.578	1.489 ± 0.222	2.502 ± 0.307
500	153.5	7.146 ± 0.388	1.816 ± 0.078	2.736 ± 0.204
<i>Ad libitum</i>	168.7	7.950 ± 0.837	2.021 ± 0.159	2.986 ± 0.362

Each value ($\bar{X} \pm \text{SD}$) represents the average performance of 6 to 9 individuals.

3. Results

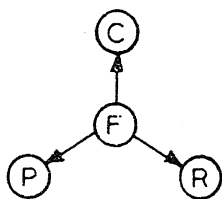
Regression equations used are provided in table 3. Most of the values of *C*, *P* and *R* predicted through simple regression equations (Nos. 1, 3 and 5; table 3) using *F* alone as the independent variable were close to the observed values. The total chi-square values for the differences between the observed values and those obtained through these equations were not statistically significant suggesting that the latter come from the population of observed data. However, comparison of the predicted values provided in table 4 with observed values in table 2 revealed that a few of the predicted values deviated widely from observed values. For instance, *C* value for the larva receiving 50 mg ration at 22°C was (0.974 kJ/g larva/day) 24% less than the observed value (1.288 kJ/g/day). Similarly, the *P* and *R* values in a few

Table 3. Regression equations used for the prediction of bioenergetics components of final instar *Achaea janata*.

Equation Number	Source of data	Regression equation	<i>r</i>	<i>N</i>
1	All temperatures and ration levels	$C = -0.2176 + 0.0459 F$	0.933	24
2	All temperatures and ration levels	$C = -0.0145 + 0.436 F + 0.00136 T$	0.984	24
3	All temperatures and highest 5 ration levels	$P = -0.2673 + 0.0134 F$	0.945	20
4	—do—	$P = 0.257 + 0.0155 F + (-0.0263) T$	0.971	20
5	—do—	$M = -0.0636 + 0.0161 F$	0.901	20
6	—do—	$M = -1.159 + 0.119 F + 0.0547 T$	0.945	20

Mean values of *F*, *C*, *P* or *R* of 6 to 10 observations at the tested temperatures and ration schedules were used for calculating the regression equations. In equations 2, 4 and 7, the numerical value of temperature (*T*) has been used as the second predictor variable.

ration schedules at 22°C and the 200 mg ration schedule at 35°C included considerable error. To improve the accuracy of estimation the numerical value of temperature (*T*) was combined with *F* as a second predictor variable and multiple regression equations were developed (Nos.2, 4 and 6; table 3). These equations used for determining *C*, *P* and *R* values significantly decreased the differences between the two sets of values as well as the total chi-square values. Therefore, it is possible to predict *C*, *P* and *R* in terms of energy from the mass value of *F* as shown by the schematic pathway given below:



4. Discussion

Bioenergetics data reported for the moth *Platysamia cecropia* fed *ad libitum* at 25, 24, 30 and 27°C (Schroeder 1972) and for the monarch butterfly *Danaus chrysippus* fed on decreased rations or for restricted durations (Mathavan and Muthukrishnan 1976) were chosen to test whether the bioenergetics components can be predicted in line with the model proposed for *A. janata*. Linear regression equations were calculated for the relation between *F* and *C*, *F* and *P* and *F* and *R* using which the bioenergetics data were determined (tables 5 and 6). The error does not exceed 5% for most of the data.

Accuracy of estimation of *C* from *F* depends on egestion of a constant fraction of consumed food as faeces. In other words, constancy of approximate digestibility or

assimilation efficiency under different experimental conditions determines the accuracy of prediction of C from F . In addition to constant assimilation efficiency, allocation of a definite proportion of digested food for growth (= conversion) and the rest for metabolism would increase the accuracy of prediction of P and R from F . Availability of feed or ration level does not alter the efficiencies of assimilation and conversion of digested food (e.g. *D. chrysippus*: Mathavan and

Table 4. Predicted values of C , P and R (kJ/g larva/day) of the final instar *Achaea janata* at the tested temperatures and ration levels (mg leaf/larva/day).

Ration	C 1	C 2	P 3	P 4	R 5	R 6
22°C						
50	0.974	1.147	—	—	—	—
100	1.673	1.812	0.287	0.317	0.600	0.535
200	2.968	3.041	0.667	0.754	1.054	0.870
300	3.854	3.883	0.927	1.053	1.364	1.106
400	4.634	4.624	1.155	1.317	1.638	1.302
ad libitum	5.465	5.413	1.399	1.597	1.930	1.518
27°C						
50	1.540	1.692	—	—	—	—
100	2.601	2.699	0.559	0.499	0.925	1.049
200	4.322	4.334	1.064	1.080	1.529	1.495
300	5.157	5.128	1.309	1.362	1.822	1.711
400	5.878	5.812	1.520	1.605	2.074	1.898
ad libitum	7.604	7.452	2.026	2.188	2.680	2.346
32°C						
100	2.738	2.837	—	—	—	—
200	4.037	4.071	0.980	0.852	1.429	1.694
300	5.134	5.113	1.302	1.223	1.814	1.979
400	5.717	5.667	1.473	1.420	2.018	2.130
500	6.566	6.473	1.722	1.706	2.316	2.350
ad libitum	7.200	7.075	1.968	1.902	2.538	2.514
35°C						
100	2.917	3.011	—	—	—	—
200	4.239	4.267	1.039	0.841	1.500	1.911
300	5.176	5.156	1.314	1.158	1.828	2.154
400	6.300	6.224	1.644	1.538	2.223	2.445
500	6.828	6.726	1.800	1.716	2.408	2.582
ad libitum	7.526	7.388	2.003	1.951	2.652	2.763
Total X^2 *	0.298	0.272	0.706	0.305	0.765	0.282

* Significance levels of total chi-square values less than 7.01 at degrees of freedom 18, fall at $P > 0.99$.

X^2 values indicate the goodness of fit of the predicted values with the observed values given in table 2. Numerals 1,2,3....6 indicate the number of equation in table 3 used for predicting the values.

Table 5. Observed and predicted values of consumption (*C*), production (*P*) and metabolism (*R*) of *Platysamia cercopia* larva fed on lilac leaves.

Temperature °C	<i>F</i>	Observed <i>C</i>	Predicted <i>C</i> *	Observed <i>P</i>	Predicted <i>P</i> **	Observed <i>R</i>	Predicted <i>R</i> ***
25.0	10.9	293.44	302.2	63.68	61.6	184.13	195.0
24.2	10.8	306.83	300.6	63.21	61.3	198.42	194.2
30.0	9.8	285.07	285.0	57.30	58.3	186.74	185.6
27.0	11.2	309.35	306.9	59.40	62.5	203.07	197.6

* $C = 131.438 + 15.666 F$; $r = 0.832$; ** $P = 29.313 + 2.959 F$; $r = 0.586$; *** $R = 102.043 + 8.529 F$; $r = 0.565$.

Values of *F* (g/larva), *C*, *P* and *R* (kJ/g larva) reported by Schroeder (1972) have been used to test the adequacy of the model proposed for *Achaea janata*. Predicted values were obtained by using the regression equations given below.

Table 6. Observed and predicted values of consumption (*C*), Production (*P*) and metabolism (*R*) of final instar *Danaus chrysippus* larva fed on restricted rations or durations.

Ration Feeding duration	<i>F</i>	Observed <i>C</i>	Predicted <i>C</i> *	Observed <i>P</i>	Predicted <i>P</i> **	Observed <i>R</i>	Predicted <i>R</i> ***
25%	127	216	217.2	35	33.1	54	57.0
50%	166	290	296.2	51	51.8	73	78.3
100%	344	651	656.4	135	137.0	172	175.4
3 hr/day	130	217	223.3	31	34.6	56	58.6
6 hr/day	150	266	263.8	43	44.1	73	69.5
9 hr/day	186	346	336.6	64	61.4	96	89.2
12 hr/day	244	461	454.0	92	89.1	125	120.9

* $C = -39.82 + 2.024 F$; $r = 0.999$; ** $P = -27.667 + 0.4786 F$; $r = 0.998$; *** $R = -12.347 + 0.5459 F$; $r = 0.994$.

Values of *F*, *C*, *P* and *R* (mg(dry)/larva) reported by Mathavan and Muthukrishnan (1976) have been used to test the adequacy of the model proposed for *Achaea janata*. Predicted values were obtained by using the regression equations given above.

Muthukrishnan 1976; *Calocalpe undulata*: Schroeder 1976; *A. janata*: Muthukrishnan 1980). However, variation in habitat temperature brings about significant changes in these efficiencies (see Scriber and Slansky 1981; Schroeder 1981) and is likely to introduce error in the values predicted from *F*. Inclusion of temperature as an independent variable with *F* may help to improve the accuracy of prediction. Development of similar linear regression models for larvae of common species of lepidoptera may help to easily assess the damage inflicted by defoliators to agricultural crops as well as in studies on energy flow through consumer species.

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Incubation, hatching and yolk utilization in the eggs of the orb weaving spider *Cyrtophora cicatrosa* (Araneae; Araneidae)

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Abstract. *Cyrtophora cicatrosa* Stoliczka was reared from hatching to death at 4 different temperatures i.e. 22, 27, 32 and 37° C. A significant correlation is obtained between initial water content and hatchability of the eggs. During embryonic development energy density and fat decreased but the water content increased. Fat was the main energy source for embryonic development. Of 0.34 gcal energy present in an egg, 29% is utilized on embryonic metabolism, 21% on postemergent activities.

Keywords. Incubation; yolk utilization; hatchability; water content of eggs; *Cyrtophora cicatrosa*.

1. Introduction

The pattern of yolk utilization is known to reflect the physiological aspect of the embryo in relation to environment (Needham 1931). The spider egg must contain enough energy and nutrients to meet the metabolic cost of embryonic development, emergence, dispersal and other post-emergent activities (Anderson 1978). Aerial dispersal by ballooning may heavily tax yolk energy reserves of a spiderling (Valerio 1977). Subsequently the spiderling must be able to establish a web, before it can draw energy from food. Unlike the young ones of crustaceans and insects, a spiderling requires an additional fraction of yolk energy to meet the post-emergent activities. Hence, the pattern of yolk utilization in a spider is likely to exhibit adaptive strategy. Yet, no studies have been reported on the efficiency of yolk utilization in spiders. But there are numerous publications on this aspect for crustaceans (Pandian 1967, 1970a, b, 1972) and insects (Mathavan 1975). The present paper reports on the incubation, hatching and yolk utilization in the orb weaving spider *Cyrtophora cicatrosa* (Stoliczka).

2. Material and methods

C. cicatrosa inhabits commonly on the fence of *Euphorbia antiquorum* in Palni (10° 23' N; 77° 31' E) areas. It lays a string of egg sacs, which are hung like a beaded chain above the orb net. The oldest egg sac is at the top and the recent one at the bottom. This facilitated the collection and separation of eggs at different developmental stages from a string of known parentage.

The rate of development, was studied at 22, 27, 32 and 37° C at each temperature, sacs containing 150 eggs were used. The desired temperature was maintained to an accuracy of $\pm 1^\circ$ C in a BOD incubator. To observe the effect of ration on incubation

and hatching, eggs were obtained by rearing the spider fed *ad libitum*, which was equivalent to 100% ration; considering maximum food consumption of adult female, other rations such as 60, 30 or 15% were formulated and thus eggs were obtained by feeding the spiders with fixed quantity of prey. The eggs were maintained under even illumination for 12 hr/day. A cotton pad soaked in water provided uniform humidity (approximately 85% RH). Hatching was observed through stereo-dissection microscope.

For chemical and calorific analyses, the following developmental stages were chosen:

- Stage I Undeveloped egg — soon after oviposition — nearly transparent — spherical with a diameter of 0.7 to 0.9 mm (age 1 day)
- Stage II Mid embryonic period — heart beats — translucent — oval in shape (age 4-6 days)
- Stage III Fully formed embryo — pear-shaped — cephalothorax, abdomen and appendages visible through chorion (age 9-11 days)
- Stage IV Freshly hatched spiderling — with plenty of yolk in opisthosoma — not yet able to spin its web — moves its appendages continuously (age 12 days)
- Stage V Young spiderling — yolk fully spent — active — able to spin its own dense snare — attained characteristic pigmentation by tanning of cuticle (age 16+2 days)

Water content of developing eggs and spiderlings was determined by weighing the test material before and after drying at 90° C to weight constancy. Organic substance was estimated subtracting ash content from dry matter. Ash was determined incinerating the test material before and after drying (about 40 mg dry substance) in a muffle furnace at 560° C for 6 hr and weighing the residue, as recommended by Paine (1964).

Fat content was estimated as the difference between the dry weight (40 mg) and fat-free dry weight of the test substance, determined after 8 hr extraction with petroleum ether in semi-micro soxhlet apparatus, according to the method detailed by the Association of Official Agricultural Chemists (1950).

The eggs and spiderlings were weighed in a single pan balance (Mechaniki model) to an accuracy of 10 µg. Calorific content of different developmental stages was determined in a semimicro bomb calorimeter (Parr Instrument Co.) following the standard procedure described in Parr Instruction Manuals No. 128 and 130. When the sample was small (15 mg), a known quantity (25 mg) of benzoic acid was added to the sample and the combined calorific value was estimated.

3. Results

In *C. cicatrosa* development proceeds uninterrupted, i.e. diapause does not occur at the tested temperatures. Once eclosion is commenced, hatching of the simultaneously incubated eggs lasts for 2 days at temperatures 27° C and 6 days at 22° C. The incubation period is extended from 7 days at 37° C to 24 days at 22° C (table 1).

A number of factors influence hatchability of the incubated eggs. Age of the female appears to be an important factor; hatchability decreased from over 90% in the first 7 egg sacs to 40% in the 16th sac (table 2). Incidentally, the eggs oviposited by aged females contain less water. In spiders exposed to different temperatures, water content

Table 1. Effect of temperature and ration (at 27°C) on incubation and hatchability in *Cyrtophora cicatrosa*.

Temperature/ Ration	Incubation period (day)	Water (%)	Hatchability (%)
37°C	7 ± 1.1	27.1 ± 6.1	2 ± 0.3
32°C	9 ± 1.3	71.1 ± 6.8	82 ± 2.9
27°C	12 ± 1.7	65.6 ± 4.3	91 ± 3.7
22°C	24 ± 3.1	65.8 ± 5.1	75 ± 5.5
100%	12 ± 1.7	64.6 ± 4.5	90 ± 3.6
60%	12 ± 1.7	69.1 ± 4.8	74 ± 5.2
30%	12 ± 1.7	62.4 ± 4.1	67 ± 3.7
15%	12 ± 1.7	50.0 ± 3.6	55 ± 4.1

Each value (mean ± SD) represents the mean obtained from 21-36 egg sacs oviposited by *ad libitum* fed females. Water content of the eggs was determined soon after oviposition.

Table 2. Effect of female age on egg hatchability.

Age of female (day)	Egg sac number	Water content (%)	Hatchability (%)
96	1	65 ± 4.3	93 ± 2.1
101	2	66 ± 5.1	96 ± 1.7
107	3	68 ± 3.6	96 ± 2.2
112	4	69 ± 3.6	92 ± 3.3
117	5	71 ± 2.5	94 ± 2.7
121	6	68 ± 3.1	91 ± 4.1
126	7	68 ± 2.9	91 ± 5.3
132	8	59 ± 6.1	88 ± 4.6
139	9	66 ± 4.2	81 ± 6.7
146	10	69 ± 2.4	67 ± 7.6
155	11	69 ± 1.9	69 ± 5.2
163	12	60 ± 4.7	65 ± 8.1
172	13	63 ± 3.8	46 ± 10.2
181	14	53 ± 4.6	40 ± 9.2
190	15	55 ± 5.3	42 ± 16.2
200	16	48 ± 6.8	40 ± 14.6

Each value (mean ± SD) represents the mean obtained from 7-12 egg sacs oviposited by *C. cicatrosa* fed *ad libitum* at 27°C

of the eggs, another important factor, progressively decreased from 65% in the eggs of first few sacs to less than 50% in those contained in the last few egg sacs. Temperature also affects hatchability, which was around 80% in eggs incubated at 22, 27 or 32°C; at 37°C it was 2% only. Similarly, the eggs oviposited by females exposed to diminishing rations contain less water and correspondingly, the hatchability of these eggs is also reduced. On the whole, a significant positive relation ($P < 0.01$) became apparent between hatchability and water content of eggs oviposited under different rearing conditions; the correlation coefficient obtained ($r = 0.779$) for this relation indicated that water content significantly affected hatchability.

Spiderlings hatched throughout the day but the peak hatchability occurred at midnight. Considering the total eggs incubated, the maximum number was hatched (40%) between midnight and 0300 hrs. A freshly hatched spiderling is completely

Table 3. Changes in chemical composition and calorific content of developing eggs/spiderlings in *C. cicatrosa*

Developmental stage	Water content	Fat	Ash	Protein	Energy
I	69 ± 8.6	40 ± 1.1	1.3 ± 0.06	58.7	6440 ± 193
II	74 ± 3.6	37 ± 0.7	1.3 ± 0.05	61.7	5995 ± 215
III	72 ± 3.6	32 ± 1.3	1.4 ± 0.06	66.6	5810 ± 189
IV	72 ± 2.9	30 ± 1.2	1.4 ± 0.07	68.6	5751 ± 196
V	77 ± 3.0	29 ± 1.0	1.4 ± 0.08	69.6	5325 ± 213

Each value (mean ± SD) represents the mean of 3-8 estimations: energy in kcal/g dry weight: others in %. Water content is given as a percentage of live weight and the values for fat, ash and protein are given as percentage of dry weight and energy in cal/g dry substance; each value (mean ± SD) represents the mean of 4 to 40 estimates.

Table 4. Average changes of wet weight and dry weight of a single egg/spiderling of *C. cicatrosa* in different developmental stages at 27°C.

Developmental stage	Number of animals	Total No. of eggs/spiderling counted	Mean live weight (μg/egg or spiderling)	Coefficient of variation
I	27	4150	168 ± 13.2	7.9
II	25	4250	191 ± 6.4	3.4
III	25	3950	195 ± 24.8	12.7
IV	22	3750	152 ± 13.5	8.9
V	36	5450	141 ± 17.3	12.3

devoid of any pigmentation and nearly transparent. It is quiescent, but begins to move its appendages steadily and continuously. These movements may help circulation of the blood. The spinning glands are not yet fully formed. Mouth parts are still incomplete; plenty of yolk material is retained in opisthosoma.

As the development proceeds from stage I to stage V, water content increased from 69 to 77% (table 3). During the corresponding period, protein also increased from 59 to 70%; ash content does not appreciably change, but fat decreased from 40 to 29%, which is also reflected in the depletion energy density from 6440 to 5325 kcal/g dry weight. This indicates that fat is utilized as the major energy source during embryonic development.

3.1 Changes in live and dry weights

Live weight of a single egg steadily increased from 168 μg in stage I to 195 μg in stage III but subsequently decreased to 141 μg in a spiderling (table 4). With advancing development, more than one-third of dry weight (20 μg) of yolk substance was used for embryonic metabolism.

3.2 Efficiency of yolk utilization

From the values reported in tables 3 and 4, average changes in chemical composition and (table 5) calorific content of a single egg/spiderling were calculated. Dry matter, organic substance, ash content and protein showed slight increase in stage III (prior to hatching) and then decreased progressively, as the development advanced.

Table 5. Chemical composition and calorific content of egg/spiderling in different developmental stages (weight in μg ; energy in gcal)

Developmental stage	Water	Dry matter	Organic substance	Fat	Ash	Protein	Energy
I	115.6	52.2	51.51	21.07	0.69	30.42	0.34
II	141.8	49.3	48.66	18.11	0.64	30.54	0.30
III	140.7	53.7	52.96	17.30	0.74	35.44	0.31
IV	109.6	41.7	41.22	12.56	0.58	28.64	0.24
V	108.5	32.4	31.95	9.44	0.45	22.63	0.17

The yolk utilization efficiency is a ratio of 'body formed/body formed + yolk used for metabolism'. In *C. cicatrosa*, the efficiency values were 62, 62, 50, 74 and 45% for dry weight, organic substance, calorific content, protein and fat respectively. The differences in the values indicate the efficiency with which the different substances of the yolk were utilized.

During development (stages I to IV), *C. cicatrosa* spent about 0.1 gcal on metabolic process; of this, the oxidation of protein contributed 10%, while fat oxidation supplied 89% energy (table 5).

3.3 Water metabolism

Water content increased $166\mu\text{g}$ in stage I to $141\mu\text{g}$ in stage III and thereafter, it gradually decreased to $108\mu\text{g}$ (stage V). Increases in water content in II and III stages may be contributed through the accumulation of metabolic water. The amount of water absorbed from the atmosphere through chorion is also included in this quantity. Decrease in water content in stage IV is due to water loss to the environment on hatching.

4. Discussion

A statistically significant correlation obtained between initial water content and hatchability of *C. cicatrosa* egg is a very significant observation. Eggs containing less than 50% water do not hatch readily, but those containing less than the critical minimum of 30% never hatch. At 37°C , the incubated eggs contained about 30% water and did not hatch at all. Usually the eggs oviposited by (i) aged females, (ii) females receiving 30% ration and (iii) females reared under the elevated temperatures ($> 32^\circ\text{C}$) contained less than 60% water. Failure to hatch and egg mortality appears to be one of the mechanisms, by which population size is regulated by extreme environmental factors such as prey scarcity and elevated temperature.

The fact that lipid serves as a major energy source during embryonic metabolism of the spider *C. cicatrosa* confirms the conclusion made for terrestrial animals in general by Needham (1931) and also confirms the results obtained for certain aquatic crustaceans (Pandian 1967; Katre 1977; Ponnuchamy *et al* 1979). The period between the hatching of the spiderling and its successful establishment involves aerial dispersion by ballooning and heavy expenditure of reserve yolk energy (Schaefer 1978; Anderson 1978). To support the metabolic requirements of spiderling during this phase, a large quantum of energy is retained (70%) at the time of hatching in *C. cicatrosa* for these post-emergent activities. Of the total energy (0.34 gcal) 29% of egg

energy is utilized on embryonic and 21% on post embryonic metabolism; the remaining (50%) goes to the constitution of the spiderling.

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Determination of acute toxicity levels of mercury to the fish *Tilapia mossambica* (Peters)

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Abstract. Toxicity of inorganic mercury to the freshwater fish *Tilapia mossambica* was demonstrated by static bioassays. Statistical analysis was used for calculating 48 hr median tolerance limit, 95% confidence limits and the slope function (S). No mortality was observed within 48 hr in mercuric chloride concentration of 0.7 mg l^{-1} although the swimming activity of the fish decreased and the rate of opercular movement increased significantly. Concentrations of 0.9 mg l^{-1} and higher proved lethal. Symptoms of mercury poisoning and safe concentrations of mercury have been discussed.

Keywords. Toxicity; mercury; safe concentrations; *Tilapia mossambica*.

1. Introduction

The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to industrial pollution. High toxicity of mercury compounds has been known since a long time (Bidstrup 1964), but their hazardous nature as pollutants of aquatic environment became a matter of concern only after a large number of deaths occurred from poisoning in areas adjoining the Minamata Bay, Japan (Ui 1971; Kurland *et al* 1960). The use of mercury in the manufacture of many products such as chlor-alkali or in electroplating processes as also in the paper manufacturing industry, enlarges the interest regarding the influence of this metal on the aquatic organisms. In developing countries many freshwater and estuarine ecosystems receive mercury released during the operation of small-scale industrial units located in coastal towns.

Tilapia mossambica, a freshwater fish, was selected for the study of toxicity of mercury, because of its easy availability and also because this species responds well to aquarium conditions and can readily be kept under a wide range of environmental conditions.

2. Material and methods

Specimens of *T. mossambica*, collected from the Goa State Fisheries Farm, were brought to the laboratory and kept in several glass aquarium tanks ($40 \times 20 \times 30 \text{ cm}$) containing aerated freshwater. The fish were obtained during January to March 1980 and acclimated to laboratory conditions for 2 weeks before the experiments began. Dissolved oxygen content of the water in the tank was always greater than

4.8 ml per litre and the temperature variations were within $\pm 1^\circ\text{C}$. The fish were fed daily during the acclimation period on dry pellets made from a mixture of prawns and wheat flour. This food was readily taken by the fish and was nutritious to maintain them in apparently healthy condition.

Bioassays were conducted in static waters by adopting the procedure of Doudoroff *et al* (1951). The fish were not fed during the bioassays and for two days before the start of the experiment. The size of the fish varied from 4.7-6.2 cm (average 5.5 cm) and their weight ranged from 2.2-3.5 g (average 2.8 g). Stock solution of the metal (mercuric chloride) was prepared and diluted to obtain the desired degrees of concentration.

Exploratory tests were run by maintaining higher concentrations of toxicity in the beginning. Progressively lower concentrations were tested to locate a critical concentration range.

Six test animals were placed in each of the test containers, with a water-fish ratio of 2 l of water per 3 g of fish. Slow aeration was provided to maintain adequate dissolved oxygen concentration. Temperature during the test period was $29^\circ\text{C} \pm 1^\circ\text{C}$.

Seven concentrations, *viz.* 0.7, 0.9, 1.1, 1.3, 1.5, 1.7 and 1.9 mg $\text{Hg}^{2+} \text{ l}^{-1}$ and two controls were used. The constituents were identical in all the test tanks except for the absence of the toxic metal in the control tanks. The test animals were transferred from the acclimation tank to the test environment within 20 min after the preparation of the experimental solutions. The fish were handled carefully to avoid any injury during their transfer. Fish mortality was observed at intervals of 24 and 48 hrs. The experiments were duplicated for various test concentrations and satisfactory reproducibility in the results was noted.

The reactions of the toxicant on the behaviour of the test fish, *i.e.* loss of equilibrium and movement of the operculum, were observed during the test period. The fish was considered dead when there was no respiratory or other movements, and no response was found to gentle prodding.

The 48 hr LC_{50} , its 95% confidence limits, and the slope function were determined following the method of Litchfield and Wilcoxon (1949).

3. Results and discussion

The test animals, within a short period of their introduction in the experimental tanks, exhibited signs of distress. The gulping of air by hanging on to the surface with the hind part of the body turned downwards was very evident even when sufficient amount of dissolved oxygen was available in water. This was, however, not at all seen in the control tanks. Visible signs of poisoning were manifested by periodic bursts of erratic swimming, rapid opercular movements, surfacing and gulping of air. Disorders of the central nervous system were observed when the fish, in the lethal concentrations, lost their sense of equilibrium and turned with their belly upward making jerky movements. Finally, they sank to the bottom before death occurred. A whitish substance was seen at the bottom of the test vessels which was not seen in the control tanks.

During the experiments, no mortality of fish was observed in the control tanks. This shows that there was no other injurious substance or condition causing the mortality of the fish except for the toxic metal introduced in the test tanks. The pH values in the experimental tanks varied from 7.5 to 7.9 during the test period. The percentage mortality of the fish in 24 and 48 hr is given in table 1.

Table 1. Dose mortality response of *T. mossambica* to mercury.

Concentration of mercury (ppm)	% Mortality after	
	24 hr	48 hr
Control	0	0
0.7	0	0
0.9	0	33.3
1.1	33.3	66.6
1.3	33.3	83.3
1.5	33.3	100
1.7	50.0	100
1.9	100	100

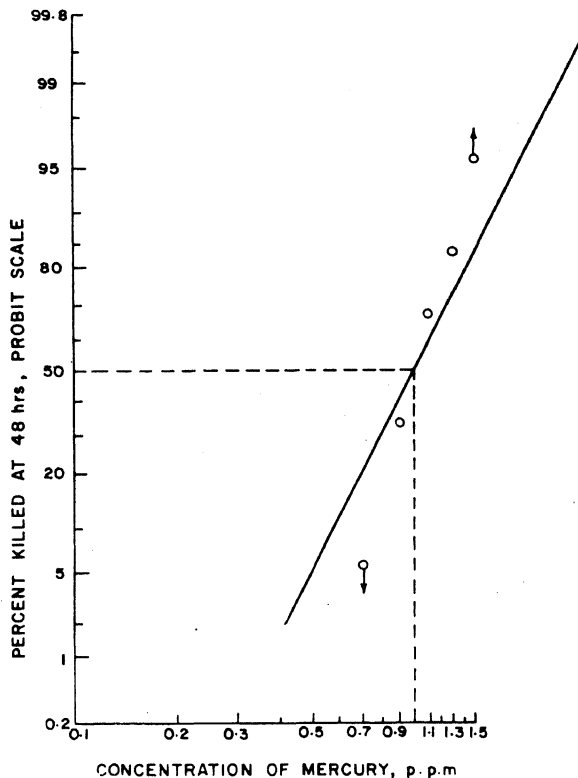


Figure 1. Dose-mortality response of mercury to *T. mossambica*.

Table 2. Toxicity of mercury to *T. mossambica* as indicated by acute toxicity tests.

Concentration of mercury (ppm)	Dead/Tested	Observed mortality (%)	Expected mortality (%)	Difference between observed and expected	Chi ² contribution (from Nomograph 1)
0.7	0 / 6	0	19	19	0.230
0.9	2 / 6	33.3	40	6.7	0.017
1.1	4 / 6	66.6	60	6.6	0.018
1.3	5 / 6	83.3	73	10.3	0.050
1.5	6 / 6	100	86	14.0	0.150

As can be seen from table 1, at 0.7 ppm of mercury, no mortality was observed within 48 hrs of the test period. However, concentrations of 0.9 and above were observed to be toxic. The 48 hr LC₅₀ value of mercury to *T. mossambica* was 1 ppm (figure 1). The 95% confidence limits were 1.261 (upper limit) and 0.792 (lower limit). The value of the slope *S* was 1.5075 (table 2).

Only limited data are available on the relative toxicity of mercury compounds to the aquatic organisms which could be compared with the present study. Jackim *et al* (1970) and Klaunig *et al* (1975) reported relatively high acute 96-hr LC₅₀ of mercury (as mercuric chloride) to adult mummichog, *Fundulus heteroclitus* of 230 and 2010 µg/l respectively. Both the studies were performed under similar laboratory conditions, utilising the fish collected during the spring and summer months. Portmann (1972) obtained a 48 hr LC₅₀ value of 3.3 ppm mercury for the fish, *Pleuronectes flesus* and between 3.3-10 ppm for the European brown shrimp, *Crangon crangon* and the European cockle, *Cardium edule*. The 24 hr LC₅₀ for the rainbow trout fingerlings was 0.9 ppm mercury (Wobeser 1975a). The above experiments were carried out at 15 and 10°C respectively. Macleod and Pessah (1973) obtained 96 hr TLm values of 0.4, 0.28 and 0.22 ppm mercury, at 5, 10 and 20°C respectively for the rainbow trout fingerlings.

Temperature has been the most important environmental factor controlling the rate of mercury uptake. The toxicity tolerance of fish decreases with an increase in temperature and fish become less tolerant to mercury in tropical conditions (Macleod and Pessah 1973; Boetius 1960). Active metabolic rate, although increases at higher temperatures, gets depressed by an exposure to mercuric chloride and higher temperatures thus augment the depressent effect indirectly (Macleod and Pessah 1973). These depressions are indicative of the damage done to the tissues of the vital systems.

In the present study, at mercury concentrations of 0.7 mg l⁻¹ and higher, the rate of opercular movement was observed to be greater than in the controls. It was also noticed that the swimming activity of the fish exposed to 0.7 mg l⁻¹ and higher concentrations decreased considerably within 20 min after the exposure. It is probable that such an increased opercular rate, associated with decreased swimming activity, compensates for the loss of efficiency in the oxygen uptake by decreasing the physiological oxygen demand and increasing the amount of oxygen passing over the branchial tissue per unit time. At concentrations of 0.9 mg l⁻¹ and above, fish were no longer able to significantly increase the rate of opercular

movement, and therefore, no further compensation could be made for the losses occurring in the efficiency of oxygen uptake. The lethality of heavy metals (lead, copper, zinc, mercury) has been ascribed to coagulation of mucus (*i.e.* precipitation of insoluble metal-protein compounds) on the gill surfaces, damage done to the gill tissues and consequently to respiratory failure (Doudoroff and Katz 1953). Increased opercular rates have been observed in fish exposed to some metals (Brafeld and Mathiessen 1976). The gills are known to accumulate significant amounts of heavy metals as compared to the other organs of the exposed fish. In conjunction with such accumulations, hypertrophy of the branchial epithelial cells has been reported in Hg, Cr, Cu, Cd and Zn toxicity (Wobeser 1975 a,b).

The symptoms of acute mercury poisoning in fish include rigidity of body, spreadout fins, slow movement of the fish and hanging on the surface with the hind part of the body turned downwards (Boetius 1960). These symptoms are followed by the loss of balance and finally sinking to the bottom before death occurs. All these symptoms were clearly visible in the test fish during the present study. One of the underlying problems with mercury pollution is the effect it provides to the nervous system by attacking the centres in the brain of human beings and other mammals (Suzuki 1969). Whether such a damage also occurs in the aquatic organisms has not been clearly demonstrated. However, similar effect seems to prevail in fish also, because some of the functions which are controlled by the nervous system such as the maintenance of equilibrium, have already been demonstrated to get disturbed with low concentrations of mercury in the fish (Lindahl and Schwanbom 1971a,b).

There is no simple formula to translate the various LC_{50} values into permissible concentrations considered safe for long periods of exposures. Although a chemical may not be directly toxic to a given fish, its cumulative action may eventually eliminate the species indirectly. In spite of these limitations in the estimation of long-term safe concentrations from the acute toxicity assays, some workers have developed certain formulae to translate the experimental data into numerical and usable values. Thus, the Ohio River Sanitation Commission (1955) has recommended an arbitrary factor of 1/10 to the 48 hr TL_m values. Hart *et al* (1945) suggested the following formula for finding out the presumably harmless concentration for the toxicant, $C = (48 \text{ hr } TL_{50} \times 0.3)/S^2$ where C = is presumably a harmless concentration, $S = 24 \text{ hr } TL_{50}/48 \text{ hr } TL_{50}$.

Many estimates made for the safe levels have been in the range of 0.1 and 0.4 toxic units. For some years, 0.1 of the 48 hr LC_{50} has been used in USA as an indicator of safe levels. Warner (1967) states that in Holland, Germany and Switzerland, application factors of 0.1 or 0.05 of the 20 day LC_{50} , which is equal to incipient LC_{50} , have been widely accepted. Recommendations for the maximum levels are 0.1 or 0.05 toxic units for the non-persistent pollutants, and 0.1-0.01 toxic units for the persistent chemicals and pesticides (Sprague 1971).

Based on the safe levels indicated above, the concentration of 0.01 to 0.04 ppm of mercury can be considered safe for *T. mossambica*.

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Aerial respiration of *Cerithidea (Cerithideopsis) cingulata* (Gmelin) and *Cerithium coralium* Kiener in relation to body weight

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Abstract. An exponential increase in oxygen consumption was observed with increase in size of *Cerithidea cingulata* and *Cerithium coralium* at 25°C. The b and $(b-1)$ values were calculated for both. The aerial regression values were compared with aquatic values.

Keywords. Aerial respiration; cerithiids; aquatic respiration; body size; *Cerithidea cingulata*; *Cerithium coralium*.

1. Introduction

Population of cerithiids form an important part of the total animal biomass of the backwater system of Bhimilipatnam, 35 km. from Visakhapatnam. Therefore, the impact of these animals on the energy and nutrient budgets of the ecosystem needs to be investigated. The present investigation is a part of the study representing the effect of body size on the aerial respiration of *C. cingulata* and *C. coralium*. Moreover, these cerithiids inhabit different regions of backwater system: *C. coralium* towards wave-swept regions and *C. cingulata* towards more exposed area. Investigations on the aquatic and anaerobic metabolisms of these cerithiids have already been reported (Prabhakara Rao and Prasada Rao 1981a,b).

2. Material and methods

The animals collected from the backwaters of Bhimilipatnam, were kept in the laboratory for 24 hr in running seawater (32%). They were not fed before and during experimentation. Respiratory measurements were made using Warburg Respirometer (B. Braun, Apparatebau, Melsungen) (Umbreit *et al* 1959) with 25 ml flasks. Potassium hydroxide solution (10%) was used to absorb carbondioxide. The bottom of the flask was wetted with Whatman 42 filtered seawater. Aerial oxygen consumption was determined at a constant temperature of 25°C. Preliminary experiments showed that the aerial oxygen consumption was fairly constant for 24 hr after acclimation of 3-4 hr. Single animal was used in each experiment. Respiratory measurements were taken every 2 hr interval over a period of 6 hr. In smaller animals, readings were taken after 6 hr and the average hourly oxygen consumption was calculated. After the experiment, the soft parts were removed after breaking the shell and dried to a constant weight at 110°C. Sixty animals were used for the experiment and they were assorted into 14 (dry weight of soft parts ranges from 11 to 167 mg) and 11 (dry weight of soft parts ranges from 8 to 73 mg) size

groups in *C. cingulata* and *C. coralium* respectively. Animals of about the same body weight were all averaged into different size groups and only the average body weight and hourly oxygen consumption were considered for the presentation of the data and statistical analysis. The allometric equation $O_2 = aW^b$ (Zeuthen 1953) was used to express the relationship of oxygen consumption to size. The regression values were calculated using the method of least squares (Snedecor and Cochran 1967). The regression lines were fitted for the calculated values and the observed values were plotted over them. For comparing the regression coefficients, t values were calculated following Student's t test (Goulden 1962).

3. Results

In both the animals, a positive correlation was obtained when oxygen consumption was plotted against body weight on a double log plot (figure 1) and a negative linear correlation was observed when the plot was drawn for metabolic rate against body weight (figure 2). Oxygen consumption increased with increase in weight in both the animals and exhibited an exponential relationship. A regression value of 0.756 was observed for *C. cingulata* and 0.8943 for *C. coralium*. The corresponding regression value for weight specific oxygen consumption is -0.244 in *C. cingulata* and -0.1057 in *C. coralium*. The former positive values represent b and the latter negative values correspond to $(b-1)$. These values with their standard errors are furnished in table 1.

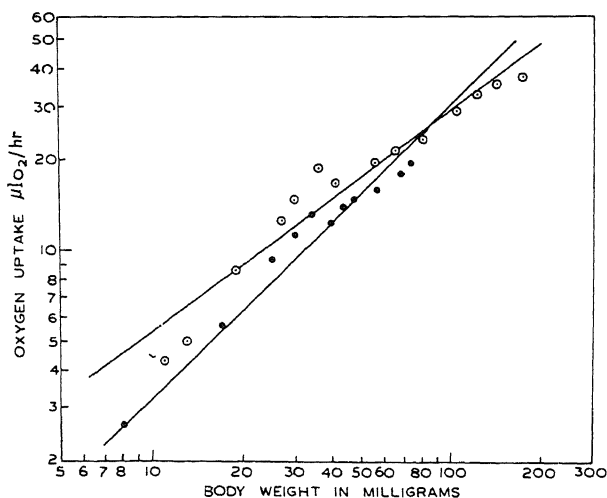


Figure 1. Relationship of oxygen consumption (aerial) and body weight in *Cerithidea cingulata* (○) and *Cerithium coralium* (●).

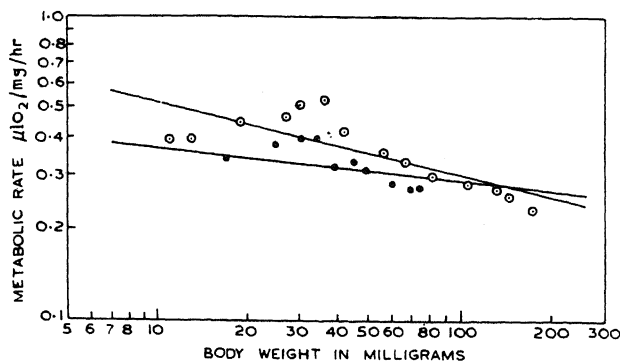


Figure 2. Relationship of metabolic rate (aerial) and body weight in *Cerithidea cingulata* (○) and *Cerithium coralium* (●).

Table 1. The coefficients of weight specific oxygen consumption of *C. cingulata* and *C. coralium* and comparison with other regression coefficients.

No.	<i>b</i>	(<i>b</i> -1)	Poikilotherms Hemmingsen: 0.75	Mollusca: 0.7	Common regression value of <i>Patella</i> : 0.6958	Aquatic values of <i>C. cingulata</i> : 0.6518 <i>C. coralium</i> : 0.7667
<i>Cerithidea</i>						
<i>cingulata</i>						
<i>r</i> : 0.9703						
<i>S_b</i> : 0.05439	14	0.7560	-0.2440	0.0060	0.0560	0.0602
				0.1103	1.0296	1.1068
				0.1000	0.1000	0.1000
<i>Cerithium</i>						
<i>coralium</i>						
<i>r</i> : 0.9802						
<i>S_b</i> : 0.05284	11	0.8943	-0.1057	0.1443	0.1943	0.1985
				2.7309	3.6771	3.7566
				0.1000	0.1000	0.1000

Under each animal is given the correlation coefficient and the standard error of the regression coefficient; Under each comparison are given the differences in the regression coefficients, the *t*-value and the probability of obtaining a *t* value greater than or equal to the observed value.

t value for *C. cingulata* and *C. coralium* at 0.1000 level of significance : 1.8238.

4. Discussion

The ability of poikilotherms to respire both in air and water was first reported by Raffy (1933) and in some of the littorines by Fisher *et al* (1933). Later aerial oxygen consumption was demonstrated in many other molluscs (Newell 1979; Houlihan 1979; Widdows *et al* 1979; Griffiths 1981). In the present investigation both the animals exhibited aerial respiration but they showed changes with the weights. It is evident that the oxygen consumption per unit time is less in smaller animals, but the metabolic rate or weight specific oxygen consumption is high for smaller ones and

less for larger ones. The aerial oxygen consumption exhibited an increase with 0.756 and 0.8943 power of the body weight and the weight specific oxygen consumption with -0.2440 and -0.1057 power of the body weight in *C. cingulata* and *C. coralium* respectively.

The b values of *C. cingulata* and *C. coralium* show a departure from the 'surface law' of Bertalanffy (1957) who proposed a quantitative relationship between metabolism and growth in various animals. A comparison of the b values of both the animals reveals that the value of *C. coralium* is significantly different ($p < 0.1$) from that of *C. cingulata* (table 1). Similar relationship has also been observed in aquatic rates of respiration of these cerithiids (Prabhakara Rao and Prasada Rao 1981a). They have reported regression values of 0.6518 for *C. cingulata* and 0.7667 for *C. coralium* for aquatic respiration. It is apparent from the comparison of aerial and aquatic regression values (table 1) that the regression values of aerial respiration are significantly different ($P < 0.1$) from that of aquatic respiration in both the animals. According to Zeuthen (1947), the b value for molluscs was 0.7 but Hemmingsen (1960) reviewed the literature on this aspect and a b value of 0.751 was proposed for poikilotherms. A comparison of the aerial regression values of both the cerithiids with that of 0.7 for molluscs of Zeuthen (1947) and 0.751 of Hemmingsen (1960), shows no significant difference ($p > 0.1$) for *C. cingulata*, while significant difference ($P < 0.1$) was observed for *C. coralium*. Davies (1966) made intraspecific and interspecific comparison of *Patella* species, *P. aspera* and *P. vulgata* from different levels and found that there was no significant difference in the effect of body weight on the metabolic rate. Therefore, a common regression coefficient of 0.6958 was adopted by him. This value (0.6958) was compared with the aerial regression values of the cerithiids. In *C. cingulata*, no significant difference was found ($P > 0.1$) but in *C. coralium*, there was a significant difference ($P < 0.1$) (table 1). However, it is clear that the regression value of *C. cingulata* is not significantly different and that of *C. coralium* is significantly different when compared with other regression values (table 1) showing species specificity as reported earlier for aquatic respiration (Prabhakara Rao and Prasada Rao 1981a).

Murdoch and Shumway (1980) measured the aerial and aquatic oxygen consumption of six species of chitons in relation to size and habitat. They observed that the regression values of aerial respiration (0.62-0.82) were found to be higher than that of aquatic regression values (0.37-0.48) in all six species. Toulmand (1967) also observed low b values in water (0.34-0.55) than in air (0.62-0.79) in several species of *Littorina*. In the present investigation, the results of high aerial regression values than that of aquatic, corroborates with those reported by Murdoch and Shumway (1980) and Toulmand (1967). They suggested that this variation might be due to their difference in the respiratory surfaces exposed to water or air.

From a comparison of aquatic (Prabhakara Rao and Prasada Rao 1981a) and aerial rates of respiration, it is clear that the aquatic rates are higher than the aerial rates. Similar increase of aquatic rates were observed in *Mytilus edulis*, *M. galloprovincialis*, *Cardium edule* and *Modiolus demissus* (Widdows *et al* 1979). Micallef (1967) stated that the ability of the species to utilise atmospheric oxygen was one of the factors to determine their distribution. Murdoch and Shumway

(1980) reported that high shore chitons had higher or equal respiratory rates in air than in water, while low shore chitons had higher aquatic than aerial rates of respiration. Therefore, from the higher rates of aquatic respiration in cerithiids, it is obvious that these animals are more adapted to aquatic respiration than to aerial respiration. In the habitat, these animals have adapted to take aerial oxygen during low tide period. Figure 1 also shows that the aerial rates of *C. cingulata* are higher than that of *C. coralium*. This might be due to its distribution: *C. cingulata* is more adapted to take aerial oxygen, because of its distribution towards more exposed areas, whereas, *C. coralium* is less adapted to aerial respiration because of its distribution towards wave-swept regions in the habitat. Similar habitat relationships were also reported by Sandison (1966) in *Thais lapillus*, *Littorina littorea*, *L. littoralis*, and *L. saxatilis*; Micallef (1967) in *Monodonta turbinata*, *Gibbula umbilicalis*, *G. cineraria* and *Callistoma zizyphinum*; Boyden (1972) in *Cerastoderma edule* and *C. glaucum*; Coleman (1973 and 1976) in *Mytilus edulis* and *Modiolus modiolus*; Bannister (1974) in *Patella caerulea* and *P. lusitanica*; Bayne *et al* (1976) in *Mytilus californianus*; Branch and Newell (1978) in *Patella cochlear*, *P. oculus* and *P. granularis*; Houlihan (1979) in *Cerithidea obtusa*, *Cassidula aurisfelis* and *Nerita articulata* and Murdoch and Shumway (1980) in six species of chitons.

Coleman (1973) observed low rates of respiration in *Mytilus edulis* during exposure to air and correlated this reduction in metabolism of *M. edulis* to the saving of energy and food reserves. However, Kuenzler (1961), Houlihan (1979) and Griffiths (1981) made it clear that aerial respiration also plays a significant role in meeting the energy demand of the animals during aerial exposure when there is the occurrence of neap tides. Further investigations on the study of energy budget of these animals during aerial exposure are in progress.

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Histochemistry of the neurosecretory systems in a stomatopod crustacean *Squilla holoschista*

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Abstract. The histochemistry of secretory material in neurosecretory cells (NSC) of *Squilla holoschista* has been investigated. The cells of eyestalk, thoracic ganglia and brain contain different substances with regard to reproductively active and quiescent phases. The NSC of eyestalk *C* and *D* are rich in protein, associated with sulphur containing aminoacids. The *B* type of NSC of thoracic ganglia is rich in lipoprotein, whereas the *C* type of the brain is glycolipoprotein.

Keywords. *Squilla holoschista*; neurosecretory cells; protein; lipoprotein; glycolipoprotein.

1. Introduction

Neurosecretory cells (NSC) were described in Crustacea for the first time by Enami (1951) who distinguished three types of cells in the brain of the crab *Sesarma*. By using mostly the paraldehyde fuchsin (PF) and chrome-alum haematoxylin phloxine (CHP) techniques, the cells were located in different nerve centres of Crustaceans (Gomori 1941, 1950). In Crustacea there have been only limited studies on the histochemistry of the neurosecretory cells. These studies were only supplementary to morphological studies in neurosecretion and have been done in decapod crustaceans. Gabe (1952a,b) observed the presence of PAS-positive material cells in the organs of Hanstrom and sinus glands in *Sphaeroma serratum*. Miyawaki (1956a,b) reported the presence of PAS-positive material in the NSCs in the crab *Telmessus cheiragonus*. Further, Miyawaki (1960a,b) reported the presence of cytoplasmic globules rich in RNA, cerebroside and phospholipids in the nerve cells of crabs. Rehm (1959) described five types of proteinaceous neurosecretory material in the crab *Carcinus maenas*. A few observations on the histochemistry of the neurosecretory systems in the entamostracan groups have been published by Barnes and Gonor (1958), Lake (1970) and McGregor (1967). Recently, studies on the histochemistry of the neurosecretory materials have increased using different histochemical techniques. However, information gathered by these methods shows some divergence. Deecaraman and Subramoniam (1983) reported the types of neurosecretory cells found in the eyestalk, thoracic ganglia and brain of *Squilla holoschista* and their cyclical activity in relation to reproductive phases. The present paper deals with the nature of the neurosecretory material elaborated by the NSC of the three centres in *S. holoschista*.

2. Material and methods

S. holoschista, which breeds all through the year along the Madras Coast, were collected and maintained in the laboratory in aquarium tanks. For the histochemical analysis, eyestalks, thoracic ganglia and brain were collected from both mature and reproductively quiescent females. The quiescent females were identified by the absence of cement glands on 6, 7 and 8 thoracic segments (Deecaraman 1980; Deecaraman and Subramoniam 1980) which is taken as an index to differentiate the mature, reproductively active females from the quiescent females. The eyestalks, thoracic ganglia and brain were fixed in Bouin's, 10% neutral buffered formaldehyde, Keley's, Carnoy and Perney's fluids. Paraffin sections (6-8 μ m thickness) were stained in paraldehyde and chrome-alum haematoxylin phloxine (Gomori 1941, 1950). Besides paraffin sections, cryocut sections were also used. The histochemical tests were made following the methods outlined by Pearse (1968), and Bancroft and Stevens (1977).

3. Results

It has been reported (Deecaraman and Subramoniam 1983) that the eyestalk consists of 4 types of NSC namely *A*, *B*, *C* and *D* and the thoracic ganglia and brain, three types namely *A*, *B* and *C*. The activities of certain NSC of the eyestalk (*C* and *D*), thoracic ganglia (*B*) and brain (*C*) show fluctuations in their secretory activity between reproductively active and reproductively quiescent stages in the synchronous growth of ovary and cement glands, suggesting their involvement in the control of reproductive activities.

3.1 Histochemical staining reaction

The tissues of different endocrine centres of NSC cells are subjected to various histochemical tests of two stages (reproductively active and quiescent stages) and table 1 denotes the chemical characterization.

The cytoplasm of different NSC cells stains purple with PF, and bluish with CHP; the NSC cells show mild differences in their staining reaction with regard to reproductively active and quiescent stages. This is evident with these two stains; however, the staining reaction with histochemical dyes are more conspicuous.

The *C* and *D* type of eyestalk NSC of reproductively quiescent state stain more intensively with acidic and basic protein stains indicating the abundance of proteinaceous substances in the secretory granules (figures 1 C,D, 4 and 5). The granules are also positive to tyrosine and sulphur containing amino acids; however, the presence of tryptophan and arginine were not detected in the granules of these two cell types. The presence of RNA in the cytoplasm is clearly shown by methyl green pyronin method. The negative histochemical reaction for oil red O, Nile blue sulphate, Sudan black, Luxol fast blue and acid haematin indicate the absence of lipid substances. Steroid is also not detected. The presence of carbohydrate substance is rather doubtful. It is therefore inferred that the neurosecretory substances synthesized in *C* and *D* type of cells are primarily proteinaceous and that they are not linked to any other prosthetic groups such as carbohydrate and lipid.

Table 1. Staining and histochemical characterization of the NSC cells of *S. holoschista*

Sl. No.	Tests and Stains	To demonstrate	Eyestalk					Thoracic ganglia					Brain		
			A	B	C	D	A	B	C	A	B	C	A	B	C
1.	Haematoxylin (Lillie 1954)	Cytoplasm and Nucleus	R	R	R	R	R	R	R	R	R	R	R	R	R
2.	Mallory's (Mallory 1938)	Cytoplasm and Nucleus	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR
3.	Masson's trichrome (Papiin 1948)	Cytoplasm and Nucleus	G	G	G	G	G	G	G	G	G	G	G	G	G
4.	Paraldehyde fuchsin (Gomori 1950)	Neurosecretory cells	P	P	P	P	P	P	P	P	P	P	P	P	P
5.	Chromalum-haematoxylin phloxine (Gomori 1941)	Neurosecretory cells	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV
6.	Ninhydrin-Schiff (Pearse 1968)	Protein bound NH ₂ groups	-	-	-	-	-	-	-	-	-	-	-	-	-
7.	Ninhydrin-Schiff after deamination		-	-	-	-	-	-	-	-	-	-	-	-	-
8.	Mercuric bromophenol blue (Maize <i>et al</i> 1953)	General and acidic blue	-	-	+++B	+++B	±	+++B	+++B	+++B	+++B	+++B	+++B	±	+++B
9.	Mercurin bromophenol blue after pepsin digestion		-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Aqu. bromophenol blue (Johri and Smyth 1956)	Basic protein	-	-	+++B	+++B	±	+++B	+++B	+++B	+++B	+++B	+++B	±	+++B
11.	Aqu. bromophenol blue after deamination		-	-	-	-	-	-	-	-	-	-	-	-	-

continued

33.	Okamoto (Pearse 1968)	Steroid	-	-	-	-	-	-	-	-
34.	Liebermann-Burchardt (Pearse 1968)	Steroid	-	-	-	-	-	-	-	-
35.	Toluidine blue PH 4.2 (Chayan <i>et al</i> 1973)	Metachromatic substances, acidic	±	+ + B	+ + B	+ B	±	+ + B	+ B	±
36.	Toluidine blue PH 5.6 (Chayan <i>et al</i> 1973)		±	+ + B	+ B	+ B	±	+ + B	+ B	±
37.	Toluidine blue (PH 4.2 & 5.6) after methylation		-	-	-	-	-	-	-	-
38.	Periodic acid Schiff:	Glycogen, 1-2 gly- col, unsaturated fatty acids and acids muco sub- stance	-	-	-	-	-	-	-	-
a.	Schiff alone (Pearse 1968)		-	-	-	-	-	-	-	-
b.	Periodic acid and Schiff (Hotchkiss 1948)		-	±	±	±	±	+ P	-	±
c.	Periodic acid after acety- lation (Pearse 1968)		-	-	-	-	-	-	-	-
d.	Periodic acid after de- acetylation (Pearse 1968)		-	±	±	±	-	±	-	±
e.	Periodic acid after dias- tase (Hotchkiss 1948)		-	±	±	±	-	±	-	±
39.	Best's carmine (Pearse 1968)	Glycogen	+	R	±	±	±	±	-	±
40.	Best's carmine after diastase		-	-	-	-	-	-	-	-

KEY: B = Blue, BG = Bluish green, BR = Bluish red, BL = Black, BV = Bluish violet, F = Faint, G = Green, I = Intense, OR = Orange, P = Pink, R = Red, $\pm$ = Doubtful, + = Positive, ++ = Moderate, +++ = Intense, -- = Negative.

* Obtained after comparing the reactions of the NSC or reproductively active and quiescent stages.

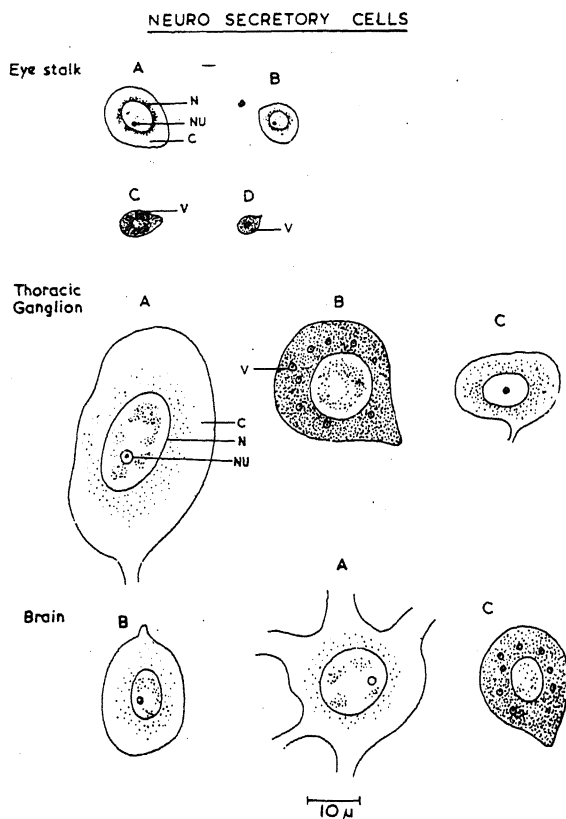


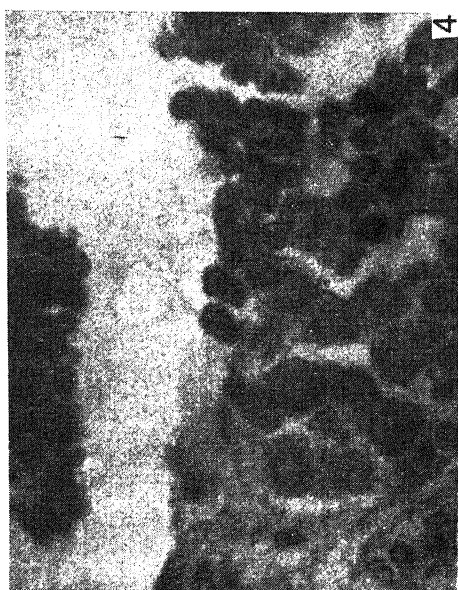
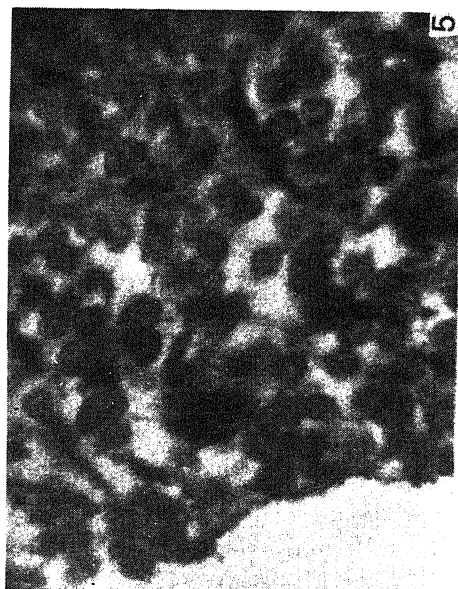
Figure 1. Camera lucida drawing showing the types of neurosecretory cells in eyestalk, thoracic ganglia and brain. (i) Eyestalk. Note the secretory activity of *C* and *D* type of neurosecretory cells at reproductively quiescent state. (ii) Thoracic ganglia. Note the active phase of *B* type of neurosecretory cell at reproductively active state. (iii) Brain. Note the active phase of *D* type of neurosecretory cell at reproductively active state.

The other types of NSC namely *A* and *B* of the eyestalk fail to show any substantial reaction to any of the histochemical tests in different phases of reproductive periods (figures 1A,B, 2 and 3).

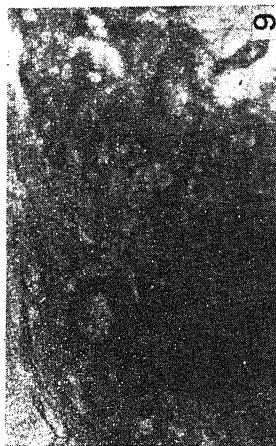
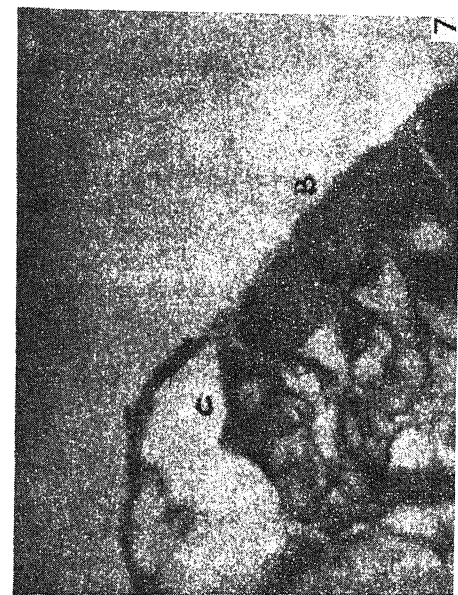
The *B* type of NSC of the thoracic ganglia also shows differences in its histochemical reaction in different phases of reproductive cycles (figures 1B and 7). This type of NSC is positive to basic and acidic proteins. Besides, the granules are positive to sulphhydryl (—SH) and disulphide (—S—S) reactive groups indicating the presence of sulphur containing amino acids. The other *A* and *C* NSC fail to show any appreciable histochemical reactions (figures 1A,C, 6 and 7).

The occurrence of RNA is also detected with methyl green pyronin test. The positive reaction for acid haematin, luxol fast blue and also for Sudan black may reveal the presence of phospholipid in the secretory granules of this type of NSC in the thoracic ganglia. The carbohydrate, however, is not detected. Evidently the secretory substance emanating from the NSC of thoracic ganglia is a lipoprotein.

Among the three types of NSC that are found in the brain, only the *C* type shows mild positive histochemical reaction to acidic and general proteins (figure 1C).



Figures 2-5. ($\times 1125$). 2,3. Neurosecretory cells at reproductively active state (stained in aqueous bromophenol blue). 2. A type 3. B type. 4,5. Neurosecretory cells at reproductively quiescent state. 4. C type (stained in chrome-alum-haematoxylin phlorine). 5. D type (stained in aqueous bromophenol blue).



Figures 6-9. (6,8,9 $\times 250$; 7 $\times 1125$). 6,7. Neurosecretory cells of thoracic ganglia (stained in aqueous bromophenol blue). 6. A type at reproductively quiescent state. 7. B and C types. 8,9. Neurosecretory cell of brain at reproductively quiescent state (stained in Ehrlich's haematoxylin). 8. A type. 9. B type.

The granules also stain for lipid as indicated by the Sudan Black dye. The PAS, toluidine blue, and Best's Carmine are also positive to these granules indicating that the secretion is a glycolipoprotein, however the concentration of the staining reaction is mild. On the other hand, the *A* and *B* NSC fail to show any significant histochemical reaction as that of *C* NSC (figures 1A,B, 8 and 9).

4. Discussion

The present results show the diverse nature of secretory substances elaborated by the different NSC located in different centres namely eyestalk, thoracic ganglia and brain.

The *C* and *D* types of NSC of the eyestalk show at reproductively quiescent state more protein moiety associated with tyrosine and sulphur containing amino acids. The presence of RNA is also detected in the cytoplasm of these cells. On the other hand carbohydrate and lipid substances are absent, suggesting that these cells secrete substances which are highly proteinaceous. This finding is in agreement with the view of Kleinholz (1966) and Adiyodi and Adiyodi (1968) that gonad inhibiting hormone in the crustacean may be in the form of a peptide or polypeptide.

The *B* type NSC of thoracic ganglia at reproductively active state also shows much secretory activity and the elaborated substance in lipoprotein associated with protein reactive groups namely S—S and —SH. On the other hand the *C* type of NSC of the brain at reproductively active state shows the presence of protein, lipid and carbohydrate substances, indicating glycolipoprotein complex.

At this point it is of interest to note in *Paratelphusa hydrodromous* (Parameswaran 1956), the presence of phospholipid in the neurosecretory cell of the thoracic ganglia. Similarly the positivity to Sudan black and also for protein-bound S—S and —SH groups have been reported in the cephalic nervous system of *Chirocephalus diaphanus* (Lake 1970) and *Rivulogammarus syriacus* (Dabbagh and Baid 1976). The presence of PAS-positive substances in the neurosecretory material have been reported in isopod (Gabe 1952a,b). Miyawaki (1958) has also made similar observation in an isopoda *Idotea japonica*. Similarly the amylase-digestible PAS positive neurosecretory granules have been recorded in one of the two types of neurosecretory cells in barnacles (Barnes and Gonor 1958).

In conclusion it may be established that the neurosecretory cells of eyestalk, thoracic ganglia and brain of *S. holoschista* contain histochemically different substances. The material of *C* and *D* cells of eyestalk is rich in protein substances. The neurosecretory material of *B* type of cells is lipoprotein and the *C* type of NSC of brain shows positivity to glycolipoprotein substances.

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Endocrine regulation of ovarian maturation and cement glands activity in a stomatopod crustacean *Squilla holoschista*

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Abstract. In *S. holoschista*, the eyestalk is provided with four types of neurosecretory cells (NSC) while the thoracic ganglia and the brain each have three types. The activities of certain NSC types of the eyestalk (C and D), the thoracic ganglia (B) and the brain (C) show fluctuations in their secretory activity during the synchronous growth of the ovary and cement glands suggesting their involvement in the control of these organs. Eyestalk ablation experiments on the reproductively quiescent female revealed the inhibitory nature of an eyestalk hormone on the ovarian and cement glands growth. On the other hand, aqueous extract of thoracic ganglia from the mature reproductive females promoted ovarian and cement glands activities, when injected into the quiescent females. But brain extract injection failed to affect the activity of the ovary or cement glands.

Keywords. *Squilla holoschista*; reproductively quiescent and active stages; secretory granules; gonad inhibiting hormone and gonad stimulating hormone; ovary; cement glands.

1. Introduction

In decapod Crustacea, the reproductive hormones fall under two categories; an inhibiting principle originating from the eyestalk and a stimulating hormone emanating from the brain, or thoracic ganglia (Adiyodi and Adiyodi 1970). They have been shown to control the gametogenic activities in a variety of crustaceans. However, their activities, if any, in the functioning of accessory reproductive glands and the reproductive tract or in co-ordinating the post ovulatory processes, have not been understood properly. Several decapods incubate their eggs externally on the appendages. In a few cases, such as the crayfish, a distinct female accessory sex gland namely the cement glands are reported to release a mucoid substance that helps in the agglutination of the egg masses. Stephens (1952a) investigated the endocrine principle regulating the cement glands and ovarian activity in the crayfish, *Procambarus* and suggested a bihormonal mechanism to regulate both activities.

Different species of stomatopods inhabiting the tropical and temperate waters have been shown to possess cement glands whose activity is similar to that of crayfishes. In *Squilla mantis* inhabiting the Mediterranean waters, cement glands activity has been shown to be synchronous with the ovarian activity (Dochi 1975). In *S. holoschista* a similar cyclical activity of the cement glands correlated with the ovarian development has been reported recently. It was also shown that the cement glands secretion is highly mucoid sulphated acidmucopolysaccharide

substance, complexed with protein (Deecaraman 1980; Deecaraman and Subramoniam 1980). Here, we report the mechanism of neuroendocrine regulation of both cement glands and ovarian activity in a stomatopod crustacean *S. holoschista*.

2. Materials and methods

Mature females of *S. holoschista* (Woodmason), collected from the Madras coast were maintained in the laboratory in aquarium. The water was changed every day and sufficiently aerated. For eyestalk ablation, mature but reproductively quiescent females were selected from the daily collection. The quiescent females are identified by the absence of distinct patches of the cement glands on the sternal plates of the 6th, 7th and 8th thoracic segments.

Eyestalk ablation was performed by removing the stalks at the base with a fine sterilized scissors. This facilitates the removal of the sinus gland and the associated neurosecretory *X*-organ completely. This was followed immediately by cauterization with a hot needle to avoid bleeding. The destalked females were not fed during the course of this experiment. Similarly in the control, either the antennae or the antennules were removed. On the 15th day, the experimental and control females were sacrificed and weighed. Subsequent to dissection, the ovary and the cement glands were blotted dry and weighed in a monopan electric balance to the nearest milligram.

From the daily collection, both the mature females and reproductively quiescent females were isolated. The thoracic ganglia were removed from the two mature females and titrated with 1 ml of doubly-filtered seawater in an ice bath. This was later centrifuged at 3000 rpm for 15 min and 150 μ l of the supernatant immediately injected into the quiescent females. The control groups received the same dosage of filtered seawater. On the 15th day, both the groups were sacrificed and the animal, ovary and cement glands were weighed.

The brain extract was similarly prepared and these animals were also treated like the previous one.

For histological study, eyestalks, thoracic ganglia and brain of quiescent and reproductively active female were fixed in Bouin's or 10% neutral buffered formaldehyde. The eyestalks were also fixed in Perney's fluid (Mahoney 1966) and later neutralized in 5% sodium sulphate solution for 12 hr. Thin sections (6-8 μ m thickness) were cut and stained in Mallory's and Masson's and haematoxylin. Sections were also treated with chrome-alum haematoxylin phloxine and paraldehyde fuchsin stains to study neurosecretory cell (Gomori 1941, 1950).

3. Results

3.1 The *X*-organ

The *X*-organ is located distal to the medulla terminalis is of a compact type having three lobes; proximal, middle and distal (figure 1). The *X*-organ appears with a characteristic shape of grape with different types of neurosecretory cells (NSC) in different lobes. These cells show variation in their staining affinity during the quiescent as well as the reproductively active periods. The *X*-organ is

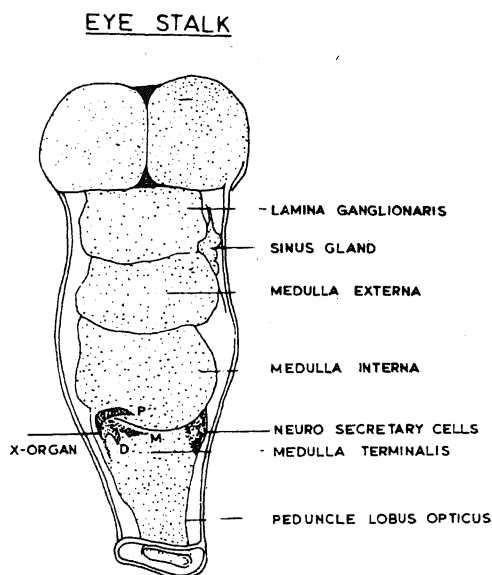
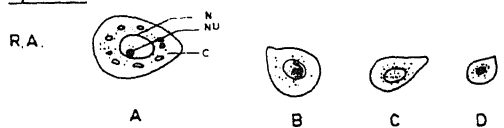
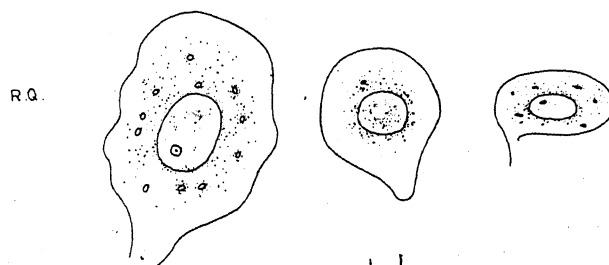
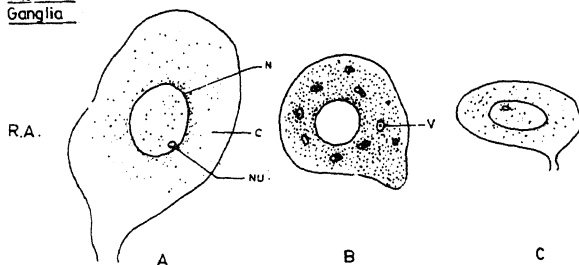
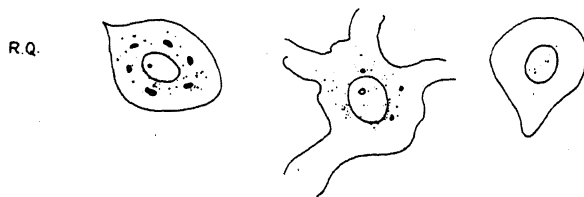
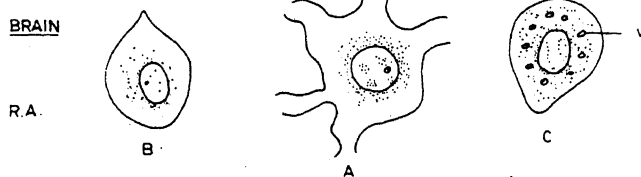


Figure 1. A composite diagram showing the *X*-organ/sinus gland complex of eyestalk with neurosecretory cells (not drawn to scale).

connected to the sinus gland by an axonal stalk called *X*-organ sinus gland tract. The *X*-organ found in the eyestalk has three types of NSC namely *A*, *B* and *D*. The *C* type of NSC are found only in the medulla terminalis. NSCs are identified and classified depending on the size and shape of the cell and nucleus, location, cytophysiological and functional behaviour.

The *A* type of NSC are pear-shaped, few in number, unipolar and provided with distinct nucleolated nuclei (figure 2). These cells measure about 12 μm and are found mostly in the distal lobe of the *X*-organ. The cytoplasm is rich with dense secretory granules only at the reproductively active period. The *B* type of NSC are spherical in shape measuring about 8 μm and are moderate in number. They are unipolar and located in the peripheral region of the middle lobe of the *X*-organ. The nucleus is spherical and without a nucleolus (figure 2). The cytoplasm is free from dense granules during the quiescent state. The *C* type is located in the medulla terminalis and are moderate in number. They are oval to elliptical in shape, unipolar and measure about 5 to 6 μm in diameter. The cytoplasm is full of secretory granules with vacuoles during the quiescent period possibly indicating an active state of secretion. The *D* type of NSC are found in the proximal lobe of the *X*-organ. These cells are unipolar, measuring about 4 μm , moderate in number and the secretory granules are rich in the cytoplasm in the quiescent period compared to reproductively active period. The presence of secretory granules in the *C* and *D* cell types when cement glands and ovaries are quiescent suggests that they may be involved in the synthesis of a gonad inhibiting hormone (GIH).

NEUROSECRETORY CELLS

EyestalkThoracic GangliaBRAIN10 μ

R.A. Reproductively active state
 R.Q. Reproductively quiescent state
 N - Nucleus, NU - Nucleolus, C - Cytoplasm, V - Vacuole

Figure 2. Camera lucida drawings showing the various types of neurosecretory cells in eyestalk, thoracic ganglia and brain at reproductively active and quiescent stages. (I) Eyestalk. Note secretory activity of C and D type of neurosecretory cells at reproductively quiescent stage. (II) Thoracic ganglia. Note the active phase of B type of cell at reproductively active state. (III) Brain. Note the active phase of C type of cell at reproductively active state.

3.2 Sinus gland

The sinus gland is a neurohaemal organ storing neurosecretion from the *X*-organ. It is located between the lamina ganglionaries and medulla externa (figure 1). The gland is somewhat triangular, having dorsal and ventral folds. Blood sinuses traverse these folds and surround the gland itself. The gland is filled with intensively staining granules during the reproductively quiescent state.

3.3 Thoracic ganglia

The thoracic ganglia are provided with three types of NSC namely *A*, *B* and *C*. The *A* type is large in size (32 μm in diameter) and hence called the "giant NSC" (figure 2). They are unipolar, few in number and located in the peripheral region of the thoracic ganglia. The nucleus is distinct with a nucleolus, which is provided with a central vacuole. The cytoplasm is filled with secretory granules only during the reproductively quiescent state. The *B* type is more or less spherical with a cone-like projection at one end and measures about 15 μm . The cells are unipolar and found towards the peripheral region of the thoracic ganglia. The cytoplasm shows more secretory granules during the reproductively active period than at quiescent state. The third type of NSC the *C* type, measures about 7 to 8 μm , and are unipolar having distinct nuclei. The cells are elliptical to oval, moderate in number and located laterally. The cytoplasm is rich with secretory granules, in the quiescent state than in the reproductively active state (figure 2). Among the three types of NSC in the thoracic ganglia only the *B* type shows more secretory activity during the reproductively active period suggesting involvement in the synthesis of gonad stimulating hormone (GSH).

3.4 Brain

The brain of *S. holoschista* is provided with three types of NSCs namely *A*, *B* and *C* (figure 2). These cells also show variation in their cyclical activity with regard to reproductively active and quiescent states. The *A* type of NSC are multipolar, 15 μm in size, few in number and found at the anterior end of the brain. The cytoplasm is rich in granules only at the quiescent state. The *B* type is oval, unipolar and located at the lateral sides of the brain. These cells measure about 10 μm , and are moderate in number. The cytoplasm shows less secretory activity during the reproductively active period than at quiescent state. The *C* type of NSC are 7 to 8 μm , unipolar and are more numerous than the other types. They are mainly distributed in the distal portion of the brain. The staining activity of cytoplasmic granules is greater during the reproductively active period than at the quiescent state.

3.5 Eyestalk ablation

The destalked females after 15 days showed a remarkable increase in the weight of the ovary and cement glands. The cement glands were in the form of lobate clusters, charged heavily with dense mucoid secretion and the ovary was yellow with mature oocytes. The mean weight and SD values of the ovary (0.2600 gm, 0.084) and cement glands (0.0375 g, 0.012) as related to the total body weight

showed a great difference to that of the control group (ovary 0.025 g, 0.012; cement glands 0.0006 g, 0.011). The statistical analysis revealed a significant increase in the proportion of (a) weight of the animal to the weight of the ovary, (b) weight of the animal to the weight of cement glands and (c) weight of the ovary to the weight of the cement glands between the control and the experimental groups (tables 1, 2A,B,C).

Table 1. Mean and standard deviation.

	Source	Weight of the ovary	Weight of the cement glands
Eyestalk ablation	Experimental	0.26 ± 0.084	0.037 ± 0.012
	Control	0.025 ± 0.01	0.0006 ± 0.011
Thoracic ganglia extract injection	Experimental	0.323 ± 0.191	0.032 ± 0.021
	Control	0.33 ± 0.020	0.0006 ± 0.0016
Brain extract injection	Experimental	0.035 ± 0.015	0.0044 ± 0.0013
	Control	0.023 ± 0.021	0.0012 ± 0.0024

(50 animals are used for each experimental and control group).

Table 2A. Summary of analysis of variance.

	Source	Observed value	Expected value at 5%
A. Eyestalk ablation	animal/ovary	15.24	Significant
	animal/cement glands	32.29	
	ovary/cement glands	81.09	
B. Thoracic ganglia extract injection	animal/ovary	26.43	3.96
	animal/cement glands	80.60	
	ovary/cement glands	52.79	
C. Brain extract	animal/ovary	0.01	Non-significant
	animal/cement glands	1.80	
	ovary/cement glands	2.76	

Note: (50 animals for experimental and control groups).

Table 2B. Test of significance (only for the experimental groups).

Source	<i>t</i> observed ovary	cement glands
Eyestalk ablation × Thoracic ganglia extract injection	2.46	0.034
Eyestalk ablation × Brain extract injection	14.13	15.60
Thoracic ganglia extract injection × Brain extract injection	11.66	3.42

T expected at 5% in all cases is 1.96.

Table 2C. Correlation (*r*) only for the experimental groups.

Source	<i>r</i>	<i>df</i>	<i>p</i>
Eyestalk ablation experiment	0.91	48	0.05*
Thoracic ganglia extract injection	0.86	48	0.05*
Brain extract injection	0.052	48	0.05 (NS)

* — Significant; NS — Non-significant.

3.6 Thoracic ganglia extracts injection

The quiescent females receiving the aqueous extract of thoracic ganglia showed an enhancement in the activities of ovary and cement glands, while the control animals did not show any such increase. A comparison of the mean weight and SD values of the ovary (0.3230 g, 0.191) and cement glands (0.0326 g, 0.0215) of the experimental group to that of the control (ovary 0.0334 g, 0.0206; cement glands 0.0334 g, 0.026) reveals the differences between these groups. This is further substantiated by the statistical analyses (tables 1, 2A,B,C).

3.7 Brain extract injection

The reproductively quiescent females that received the brain extract failed to show any significant development of the cement glands and ovary. The mean weight and SD values of ovary (0.0350 g, 0.01566) and the cement glands (0.0044 g, 0.0013) of the experimental group, when compared to the control values (ovary 0.0238 g, 0.0218; cement glands 0.0012 g, 0.0024) fails to show much difference. These differences were also statistically insignificant (tables 1,2A,B,C).

4. Discussion

Many of the malacostracan crustaceans brood their eggs in their appendages. Oviducal secretions are the secretions from the tegumental glands, which help in the agglutination of the eggs as well as in the attachment of the eggs to the pleopods. In stomatopods such secretions are produced by the distinct cement glands, the chemical composition of which has been shown to be an acid-mucopolysaccharide-protein complex and is protective in function (Deecaraman and Subramoniam 1980).

As against the information available on the hormonal control of ovarian function in Crustacea, very little is known about the accessory reproductive glands (Subramoniam 1981). Available information, however, indicates that the activities of these glands are controlled by the same reproductive hormone that influences the gonadal activity. For example, Stephens (1952a) showed in the crayfish, by the classical eyestalk ablation methods and the implantation of brain, that the cement gland activity is not only parallel to ovarian development but also controlled by the same hormones regulating the ovarian development. The results now reported corroborate the view of Stephens (1952a). Of the NSCs present in the eyestalk of

S. holoschista only types *C* and *D* exhibit cyclical fluctuations in their secretory activity correlated to cement gland and ovarian development suggesting that they may be directly involved in the inhibition of the activity of these organs. These NSC cells may be homologous to the *B* type of cells described by Matsumoto (1958) and *E* type of cells by Adiyodi (1967) involved in the production of GIH in brachyurans. Eyestalk ablation studies in *S. holoschista* now support this hypothesis and suggest the presence of inhibitory hormones in the eyestalk for gonad as well as cement glands activity. Similar results have also been reported in the cement glands activity of crayfish (Stephens 1952a).

A survey of NSC types that are distributed in the thoracic ganglia and brain has also indicated that certain cells types show cyclical variation in *S. holoschista*. The *B* and *C* types, respectively of the thoracic ganglia and the brain show cyclical variations in their secretory activity suggesting that they may be involved in the synthesis of gonad as well as cement glands stimulating hormones. The *B* and *C* types of NSC of the thoracic ganglia and the brain of *S. holoschista* may be homologous to the *A* and *A'* cells of thoracic ganglia and *A* and *E* cells of brain of the brachyuran crab, which have been implicated with the release of GSH (Matsumoto 1958, 1962). However, injection of aqueous extracts of the thoracic ganglia and brain indicates that only those animals which received thoracic ganglia extract showed acceleration in the growth rates of the ovary and cement glands. In this connection it is of interest to compare the data of Stephens (1952b) who found that the brain along with the circumoesophageal connectives induce ovary and cement glands in the crayfish. That the thoracic ganglia contains the NSC responsible for the secretions of GSH in a number of crustacean species has been indicated in the work of Otsu (1963) and Matsumoto (1958, 1962). Generic differences may thus exist in the distribution of NSC in the central nervous system for controlling the activities of both ovary and cement glands in Crustacea (Subramoniam 1981). The present data on the neuroendocrine coordination of the ovarian as well as cement glands function further high-lights the importance of the synchronous activity of these two organs. Furthermore, the present observation confirms the earlier reports on the regulation of accessory gland by the gonadotrophic hormone.

Acknowledgements

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Inheritance of resistance to malathion in *Tribolium castaneum* (Herbst)

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Abstract. Studies on the inheritance of resistance to malathion in an originally field collected malathion-resistant strain of *T. castaneum* by making genetic crosses between the resistant and a susceptible strain revealed that resistance in this strain is controlled by an autosomally inherited single major gene which is incompletely dominant.

Keywords. Resistance; malathion; *T. castaneum*.

1. Introduction

The development of resistance to insecticides in field populations of stored product pests is being increasingly reported from various parts of the world. The FAO global survey report (Champ and Dyte 1976) showed that the resistance in rust red flour beetle, *Tribolium castaneum* was of world-wide occurrence. Malathion resistance in *T. castaneum* was reported earlier from India by Bhatia *et al* (1971). In spite of its wide prevalence, there is little information regarding the inheritance of malathion resistance in this species. The present investigations were, therefore, undertaken with a view to finding the nature of inheritance of resistance to malathion in *T. castaneum*.

2. Material and methods

The malathion resistant (*R*) strain of *T. castaneum* employed in the present studies was initially collected from the field (Bhatia *et al* 1971) and reared in the laboratory for six generations under malathion pressure. A portion of the emerging adults was subjected to treatment with malathion 10% (impregnated filter-papers) and survivors picked up to start 40 single pair colonies. Pure malathion-resistant colonies were isolated by subjecting the single pair colonies in the second generation to a discriminating dose of 1% malathion (a dose which gave 100% kill in susceptible and no kill in *R* individuals) and those showing no mortality were pooled to rear the resistant strain used in the present studies. A portion of this strain was reared for two generations without insecticidal pressure and the tests carried out showed that it maintained the same level of resistance to malathion. Similarly, a susceptible strain (*S*) was isolated from single pair colonies obtained from a laboratory culture reared simultaneously without any insecticidal pressure.

The general rearing of the test insect in wheat flour consisted of the method given by Bhatia and Pradhan (1968).

2.1 Bioassay technique

The toxicity to malathion was measured by employing the impregnated filter-paper method recommended by FAO (Anonymous 1970) which consisted of exposing the adults in 5 cm diameter glass rings to Whatman No.1, 7 cm filter-papers impregnated with scalar concentrations of malathion in a 3:1:1:2 mixture of petroleum ether (60-80°C b.p.), acetone, Risella-17 oil and dibutyl phthalate. A complete test comprised three replicates of six to ten concentrations and a control. Ten to 40 adults of two weeks age were used after 1 hr starvation in each treatment, the number being constant in any one experiment. The exposure time was 24 hrs at $28 \pm 1^\circ\text{C}$ at the end of which mortality was recorded.

2.2 Genetical techniques

Initially, base line toxicity data were obtained for malathion to both the *S* and *R* strains. Reciprocal mass crosses between *S* and *R* were performed to provide sufficient off-spring for testing and for further crosses. Twelve randomly selected freshly emerged adults of each sex (sexed in the pupal stage) were utilized per cross. The adults of each cross were released in jars containing wheat flour and after 4-6 days of oviposition were sieved out and transferred to fresh jars. The newly emerged adults were used either for bioassay tests or for crossing. The F_1 , F_2 and back-cross progenies were thus reared in untreated flour and their susceptibility to malathion was determined.

2.3 Statistical analysis

The log concentration-probit mortality ($Ld-p$) lines were determined for parental, F_1 , F_2 and back-cross populations and LC_{50} values calculated, only for parental and F_1 population, by probit analysis (Finney 1952). The expected F_2 segregation on the basis of single factor Mendelian inheritance was calculated by formula given by Georghiou (1969) $X(F_2) = a_1(S) 0.25 + a_2(SR) 0.50 + a_3(R) 0.25$, where X is the expected response of F_2 to a given dose, and a_1 , a_2 and a_3 are the observed responses of *S*, *SR* and *R* populations to that dose, read from their respective regression lines. Similarly, the expected segregations of the back-crosses were calculated as: back cross to *S* parent, $X(BC) = a_1(SR) 0.50 + a_2(S) 0.50$; back-cross to *R* parent, $X(BC) = a_1(SR) 0.50 + a_2(R) 0.50$. The agreement of the observed response to the expected was tested by the χ^2 method given by Finney (1952) as $\chi^2 = (r - np)^2 / np(1 - P)$ where, r = actual number affected, n = total number treated, p = expected proportion affected.

Significant deviations of the observed from the expected are indicated by $\chi^2 > 3.8$ for 1 degree of freedom at 0.05 *P* level.

2.4 Presentation of data

In all instances reciprocal crosses were made. Since the results were found statistically similar only results from one set of the crosses have been presented.

3. Results and discussion

The toxicity data of malathion to parents and the respective crosses are summarised in table 1. A comparison of the LC_{50} values of the parents shows that the *R*-parent is 30.73 times resistant to malathion than the *S*-parent. Based on the LC_{50} values, the resistance ratios of the reciprocal F_1 's over the *S*-parent are 11.05 and 10.26 times. The LC_{50} values of F_1 from the reciprocal crosses are statistically similar, implying thereby that the resistance ratios of the two F_1 's do not differ significantly.

The statistical agreement between the LC_{50} values of the reciprocal F_1 's shows that there is no maternal effect and evidence of sex-linkage. Therefore, the character of resistance is autosomal, being transmitted to the off-spring by either the male or the female parent and no significant extrachromosomal (cytoplasmic) inheritance of resistance is involved. Further, susceptibility of the *S*, *R* and F_1 populations shows that the LC_{50} for F_1 is different from either of the parents. The resistance ratios for the *R* and the F_1 's were 30.73, 11.05 and 10.26, respectively and according to Georghiou (1969) this indicates incomplete dominance of the character. It is also revealed from figure 1 wherein the regression lines for F_1 fall in between those for the two parents.

The various log concentration-probit mortality points for the F_2 population when plotted and connected, the resultant line showed a tendency for deflection in the range of 20 to 50% mortality giving rise to the formation of distinct plateaus in the line (figure 1). The appearance of such deflections and plateaus in the $Ld-p$ line indicates that the F_2 population is segregating into three classes of phenotypes and consequently resistance may be due to a major factor. Assuming that a major factor is involved in this malathion-resistant strain and that the factor segregates in the usual Mendelian 1:2:1 ratio in the F_2 population, the observed mortalities were

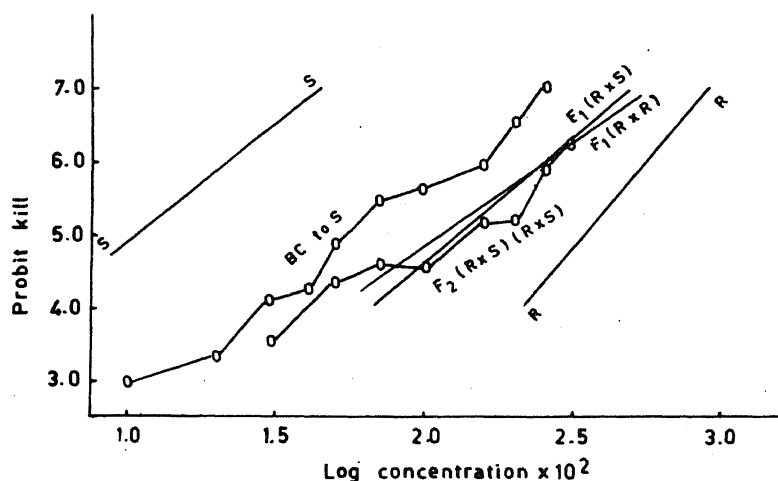


Figure 1. $Ld-p$ lines for malathion applied to the adults of susceptible (*S*) and malathion-resistant (*R*) parents and their various crosses of *T. castaneum* (straight lines have been drawn for parents and F_1 populations whereas for F_2 and back cross (*BC*) to *S*-parent points have been connected to show the deflection in the lines).

Table 1. Susceptibility to malathion of parents and the population of various crosses of malathion resistant (*R*) and susceptible (*S*) *T. castaneum*.

Malathion Conc. %	<i>S</i>	<i>R</i>	<i>F</i> ₁		<i>F</i> ₂		Back crosses			
			<i>R</i> × <i>S</i>	<i>S</i> × <i>R</i>	<i>R</i> × <i>S</i>	<i>R</i> × <i>S</i>	$\frac{(S \times R) \times S}{O \quad E}$		$\frac{(R \times S) \times R}{O \quad E}$	
					<i>O</i>	<i>E</i>	<i>x</i> ²	<i>x</i> ²	<i>O</i>	<i>x</i> ²
0.05	11									
0.10	51							03 23	6.65*	
0.15	65							—	—	
0.20	79							17 42	7.62*	
0.25	88							—	—	
0.30	99							—	—	
0.40					7	25	7.73*	27 48	5.62*	
0.50					—	—	—	—	—	
0.70					24	30	0.63	37 53	3.32	
1.00					33	36	0.17	60 57	0.10	
1.50					31	45	3.65	70 72	0.06	
2.00					56	61	0.48	77 82	0.55	26 21 1.41
2.50		14			58	67	1.89	87 88	0.04	41 35 1.68
2.75		25			73	87		100 92	2.57	47 46 0.01
3.00		36			90					62 57 1.00
3.50		48			—	—	—	—	—	—
4.00		—			92	91				—
5.00		65						89 80	2.14	66 66 0.00
6.00		73						96 87	3.17	—
		95								80 79 0.06
LC ₅₀	0.109	3.380								
Fudicial	0.084	3.210	1.205	1.119						
Limits	0.140	3.540	1.096	1.009						
Slope (b)	3.2640 ± 0.2615	4.5640 ± 0.3220	3.3220 ± 0.2860	2.8542 ± 0.2433						
<i>x</i> ²	3.02	4.71	6.88	3.72						
DF	3	4	4	4						
R ratio	—	30.73	11.05	10.26						

*Significant at $P=0.05$ for 1 DF, *O*=observed mortality, *E*=expected mortality.

analysed for goodness of fit to monofactorial inheritance. The results show a good agreement and the observed response fits well with the expected in 8 out of 9 concentrations. Hence, it is concluded that malathion resistance is controlled by a major single factor.

The $Ld-p$ line for the test-cross population shows a tendency for deflection around 50% mortality (figure 1) which suggests a segregation of groups of phenotypes in the population. The χ^2 -analysis of the observed and expected mortalities on the basis of 1:1 segregation at each concentration for the test cross population reveals that there is a close agreement between the two (table 1). However, some deviations in the range of mortality lower than 50% were observed. Similar agreement at each concentration has been found for the population of back cross to *R*-parent. This agreement in test-cross and back cross to *R*-parent further supports the conclusion that a major single factor is involved in the inheritance of resistance to malathion in this strain.

Further, the absence of overlapping in the regression lines for *SS* and *SR* genotypes suggests that a discriminating concentration of 0.5% malathion separates the susceptible phenotypes (*SS*) from that of the heterozygotes (*SR*). Based on this, at 0.5% concentration (table 2) the two F_2 populations showed 24 and 27% mortalities whereas the back crosses to *S*-parent showed 44, 60, 52 and 49% mortalities having a good fit to monofactorial inheritance by χ^2 method which also reveals that a major single factor is involved in the inheritance of malathion resistance in this strain.

In F_2 and back crosses to *S*-parent significant deviations were observed in certain lower concentrations. 0.3% concentration expected to give 25% mortality in F_2 and 0.4% and below expected to give mortalities ranging from 53 to 22% in the back cross to *S*-parent, significantly lower mortalities distorted the segregations wherever the homozygous susceptible genotypes were expected to appear. These deviations may be attributed to the elimination of susceptible genotypes due to a reduction in egg hatch (3.11%) and larval survival (11.93%) in the susceptible strain as compared to the resistant as observed by authors from the biology studies (unpublished data).

Table 2. Use of discriminatory concentration of 0.5% malathion to the adults of different populations of crosses between malathion-resistant (*R*) and susceptible (*S*) *T. castaneum*.

Cross	Total no. of insects treated	% of insects		Agreement of observed and expected frequency $\chi^2 = (O-E)^2/E$
		Survived	Dead	
$F_2(R \times S) \times (R \times S)$	45	76	24	0.63
$F_2(S \times R) \times (S \times R)$	90	73	27	1.17
$F_1(R \times S) \times S$	45	56	44	0.59
$S \times F_1(R \times S)$	60	48	52	0.21
$F_1(S \times R) \times S$	30	40	60	0.10
$S \times F_1(S \times R)$	60	51	49	1.88

All values of χ^2 are not significant at 1 degree of freedom and 0.05 level of probability as $\chi^2 < 3.84$.

Thus, the results of the present studies on the inheritance of malathion resistance in *T. castaneum* show that it is inherited as an autosomal, incompletely dominant major factor. The evidence is mainly based on (i) the absence of continuous distribution of the character of resistance in the F_2 and back cross progenies and (ii) the statistical agreement of the observed responses to those which may be expected in the case of monofactorial inheritance.

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Condition and relative condition cycles in the tropical glassy perchlet *Chanda* (= *Ambassis*) *commersonii* (Cuv. and Val.) (Pisces: Centropomidae)

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Abstract. The seasonal condition (K) and relative condition (K_n) cycles and their changes with the growth of the fish *Chanda commersonii* (Cuv. and Val.) for 2 yrs are presented. Better K is shown by the smaller immature and first maturity stages and larger, almost senile groups but they show a K_n factor below '1'. The actively breeding adults show a uniform fall in K but they give comparatively high K_n values (above '1'). These changes in K and K_n with the growth of the fish are clearly reflected in their seasonal cycles also. K cycle would thus appear to follow a pattern of build up and loss of body resources, indirectly following the breeding cycle while the K_n cycle follow the breeding and feeding cycles directly, as shown by their seasonal changes and changes with growth of the fish for 2 yrs. Since only minor deviations from unity occur in K_n factor it furnishes a more sensitive index of somatic difference between age groups, of breeding season and feeding cycle.

Keywords. Condition factor; relative condition factor; breeding cycle; feeding cycle; maturity stages; *Chanda commersonii*.

1. Introduction

Chanda (= *Ambassis*) *commersonii* (Cuv. and Val.) is a medium sized shoaling predator (Nair and Nair 1981) inhabiting the estuarine, brackish and low saline tracts of Kerala along the South-west coast of India.

Variations from the expected weight for length of individual fish or groups of fish as indicative of fatness, general well-being or gonad development may be termed as 'condition' (Le Cren 1951). Kestevan (1947) has discussed the importance of variations in specific gravity of fish flesh in studies on condition. Usually the density of the fish is maintained as same as that of the surrounding medium and hence changes in weight for length are due to change in form or volume and not specific gravity. Such changes are analysed by the K factor or coefficient of condition or ponderal index (Hile 1936; Thompson 1943) and is given by the formula $K = 1000 W/L^3$ where K = condition factor, W = weight of fish and L = length of fish, when the cube relationship with an ideal fish holds good in the length-weight relationship.

In case the fish does not obey the cube law in its length-weight relationship the relative condition factor (K_n) could be calculated using the formula $K_n = W/W$

where W = observed weight and $\bar{W}$ = calculated weight of the fish (by using the empirically calculated length-weight relationship formula).

Innumerable studies have been conducted on K cycle in fish since difference in values of condition can frequently yield insight into the circumstances of the fish's lives i.e., with regard to timing and duration of breeding cycle, food supply etc. The values of these factors to fishery science is, therefore, considerable.

With the object of tracing the K cycle of the fish, *Chanda commersonii* a commercially important species, and to find out its relationship with the growth of the fish and the breeding and feeding cycles, the monthly condition of the fish for 2 yr (December 1976–November 1978) and the change in condition with the growth of the fish were worked out.

2. Materials and Methods

The monthly collections were made from the Veli lake and Panathura estuary (Trivandrum, Kerala). A total of 250 females and 110 males (360 fish) for the first year and 310 females and 177 males (487 fish) for the second year were used for the computation.

For individuals showing isometry in length-weight relationships (females during the first year and males during the second year) the K factor was calculated for each month's samples to illustrate the seasonal cycles in condition, and for different length classes to illustrate the change in K with growth. For individuals showing departures from isometry (males during the first year and females during the second year) the corresponding K_n was calculated for each month's samples and for different length classes.

3. Results and discussion

The females range in size from 6.1–11.7 cms SL (class intervals 0.56 cm) for the first year and from 4.8–12.0 cm (class intervals 0.8 cm) for the second year. The males range in size from 5.8–8.6 cm SL (class intervals 0.31 cm) for the first year and from 4.8–8.6 cm SL (class intervals 0.33 cm) for second year.

The length-weight relationship of males and females for the two year period together with the results of the 't' test are given in table 1.

Table 1. Length-weight relationship of males and females of *C. commersonii* for 2 yrs (December 1976–November 1978)

Sex	Period	Regression equation	't' value
Female	Dec. 76 – Nov. 77	$\log Y = 2.9763 \log X - 1.5546$	0.5661 (df=248)
	Dec. 77 – Nov. 78	$\log Y = 3.1305 \log X - 1.6782$	3.2434 (df=308)
Male	Dec. 76 – Nov. 77	$\log Y = 2.8793 \log x - 1.5020$	3.8827 (df=108)
	Dec. 77 – Nov. 78	$\log Y = 2.9455 \log x - 1.5402$	1.6278 (df=175)

3.1 Relation with growth of fish

The changes in K (females during first year and males during second year) and K_n (females during second year and males during first year) with the growth of the fish are illustrated in figures 1 and 2.

The changes in K of females during the first year and males during the second year in different size classes show almost a similar gross picture. Better K is exhibited by the immature and first maturity stage females (6.1-8.34 cm SL) and immature males (4.8-6.32 cm SL). In actively spawning adult group (8.34 cm to 11.14 cm for females and 6.32-8.22 cm SL for males) almost uniformly low K is shown whereas it goes up in the largest group sampled (11.14-11.70 cm SL for females and 8.22-8.60 cm SL for males). The active breeding or spawning stress almost constantly encountered by the adult groups may be the cause for their uniform fall in K , while the immature smallest groups and almost senile largest groups show better condition due to their non-entry and cessation or slowing down in the breeding activity respectively.

The prespawners and first maturing males and females were found to have fat deposition on the immature and ripening gonads. They were found to be completely used up in ripe and actively breeding adult fishes. Qasim (1957) suggested that the waxing and waning of the K factor can probably be due to the building up or loss of reserves of the fish.

The near uniformity in the K factor shown by both adult females and males

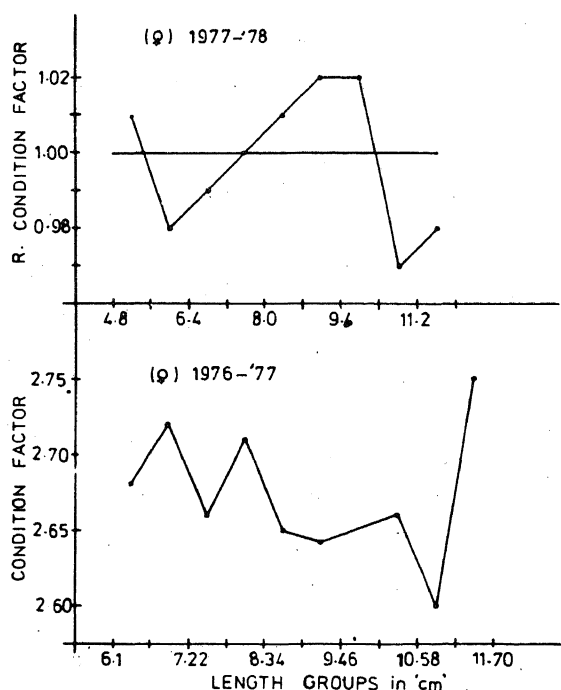


Figure 1. Variations in the mean condition (first year) and relative condition (second year) in different size groups of females of *C. commersonii*.

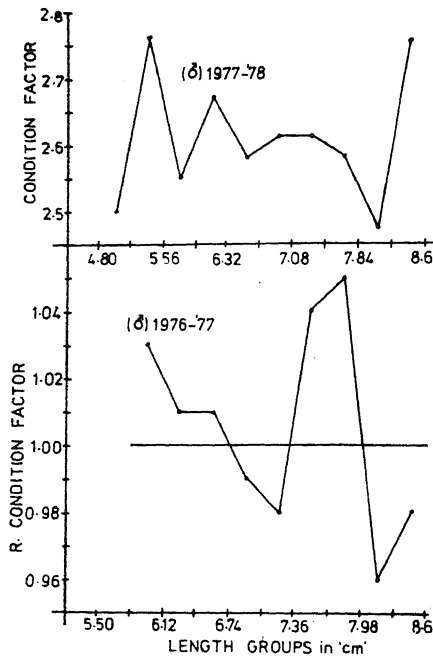


Figure 2. Variations in the mean relative condition (first year) and condition (second year) in different size groups of males of *C. commersonii*.

(different size groups) may be due to the almost year round breeding activity exhibited by the population giving only small intervals for recrudescence and subsequent spawning in the individuals.

The K_n factor for females during the second year and males during the first year show a similar gross picture, one quite different from that given by the K factor. The immature males (5.81-6.74 cm SL) show a K_n value '1' while the immature and first maturity stages of females and first maturity stages of males show a K_n value below '1'. The actively breeding groups of males (7.36-7.98 cm SL) and females (8.0-10.4 cm SL) show comparatively very high K_n values while the highest groups (7.98-8.6 cm SL for males and 10.4-12.0 cm SL for females) show comparatively very low K_n values.

Thus while the immature first maturity stages and almost senile groups show values below '1' the actively breeding adult-fish show high K_n values. Raman *et al.* (1975) made similar observations in *Ambassis gymnocephalus*, where the males and females in advanced stages of maturity showed very high K_n .

3.2 Relation between K and breeding cycle

The K cycle and gonadosomatic indices (GSI) for females (first year) and males (second year) are illustrated in figures 3 and 4. The change in K with the growth of the sexes *i.e.*, the immature and prespawners showing better K than the actively breeding adult fish is clearly reflected in the cyclic seasonal changes in K with breeding cycle also. The peaks in K factor of the population clearly alternate with the peaks in GSI for both males and females.

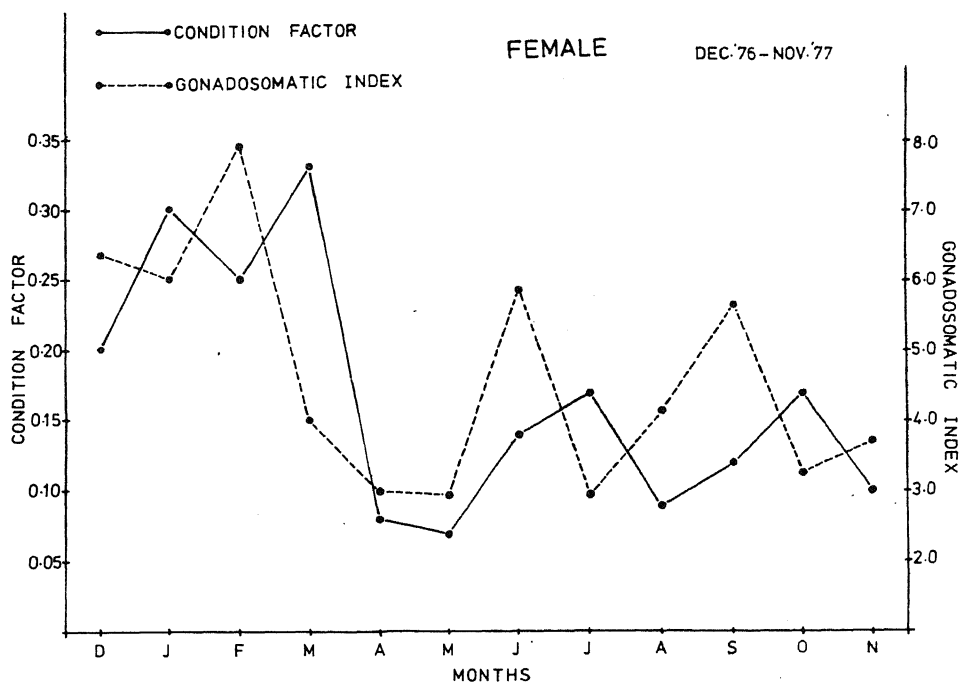


Figure 3. Relationship between seasonal cycle in condition and gonadosomatic index in females of *C. commersonii* (Dec. '76 - Nov. '77).

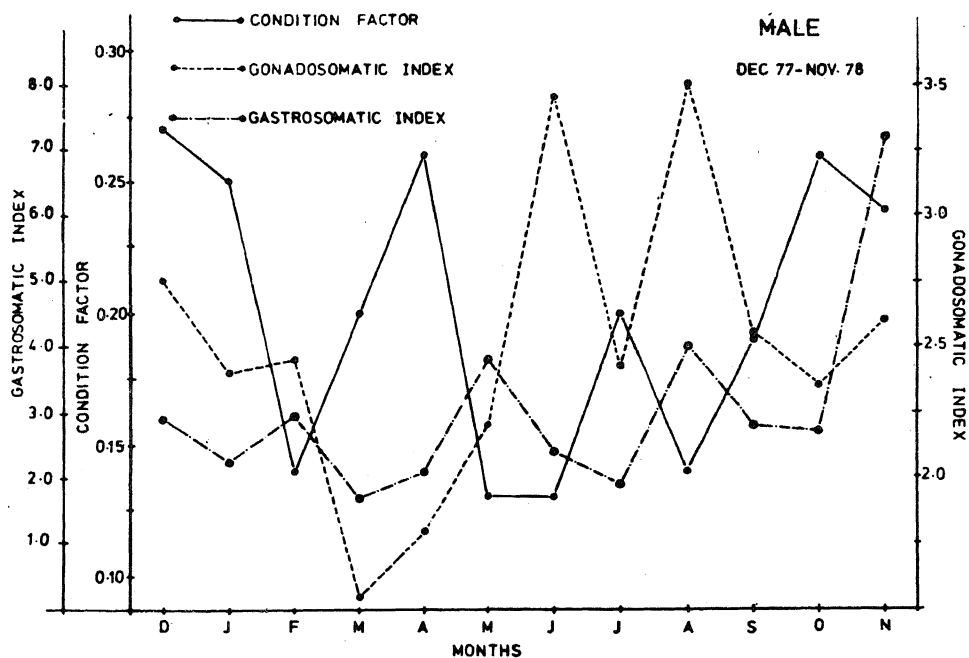


Figure 4. Relationship between seasonal cycle in condition, gonadosomatic index and gastrosomatic index in males of *C. commersonii* (Dec. '77 - Nov. '78).

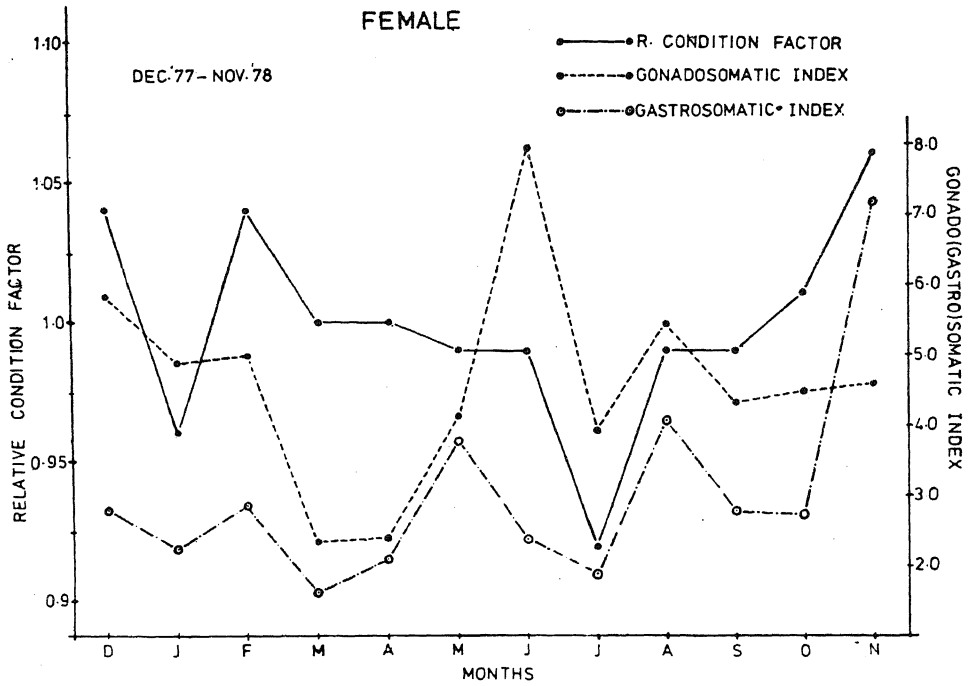


Figure 5. Relationship between seasonal cycle in relative condition, gonadosomatic index and gastroscopic index in females of *C. commersonii* (Dec. '77 - Nov. '78).

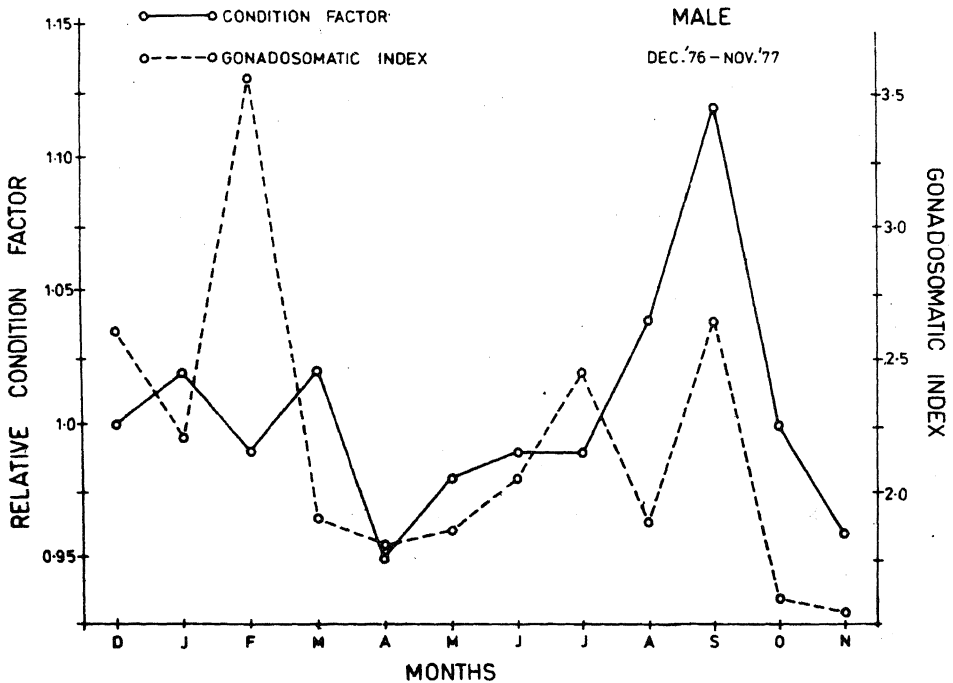


Figure 6. Relationship between seasonal cycle in relative condition and gonadosomatic index in males of *C. commersonii* (Dec. '76-Nov. '77).

Thompson (1943) pointed out that the high and low K in the plaice *Pleuronectus platessa* are found before and after spawning. Hickling (1945) in the cornish pilchard, *Sardinia pilchardus* found the condition low before spawning and high after, which was explained by him as due to sexual cycle and the availability of food respectively.

The K_n cycle and GSI for females (second year) and males (first year) are illustrated in figures 5 and 6. Similarly the changes in K_n with the growth of sexes *i.e.*, the immature and first maturity stages showing poor K_n while the adult active breeders showing better K_n is clearly reflected in the seasonal changes in K_n with breeding cycle also. Here the rise and fall in GSI is closely followed by the K_n factor, peaking and subsiding with the GSI as can be seen in the figures. Le Cren (1957) investigated K_n throughout the year in the windermere perch population in which the main difference between the patterns of change in mature males and females and immature fish were attributable to the sexual cycle — *i.e.*, change in gonad size or GSI.

3.3 Relation between condition and feeding cycle

The seasonal changes in K_n of females and the K of males for the second year are compared with the GSI in figures 4 and 5. The K_n cycle of the females closely follows the seasonal pattern taken by the GSI, the periods of heavy feeding activity showing comparatively high K_n and vice-versa. But the K cycle of males does not give any clear correlation with the feeding cycle.

4. Conclusion

Weatherley (1972) outlined a debate concerning the relative merits of calculating K as compared to that of calculating K_n . From the results of the present study the K cycle would thus appear to follow a pattern of build up and loss of body resources, indirectly following the breeding cycle, while the K_n cycle follow the breeding and feeding cycles directly, as shown by their seasonal changes and changes with the growth of sexes for the two years. Since only minor deviations from unity occur in K_n factor in this species, it furnishes a more sensitive index of somatic differences between age groups, of breeding season and feeding cycle in *C. commersonii*.

Weatherley (1972) also states that $K = W/L^3$ cannot yield a K factor very near unity for all species or even for all ages of a particular species, while a K_n factor near unity can be derived for any group of fishes, populations, age groups etc., by use of $K_n = W/aL^n$ in which case $n \neq 3$ but is derived empirically for such group. Similarly Craig (1977) is of opinion that K factor may be adequate for a simple comparison but, if used at all, for a scientific collation between population in time and or space, the K factor must be based on the length-weight relationship (K_n factor), so that any size of fish may be compared.

Acknowledgement

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Sand bathing behaviour of Indian gerbil *Tatera indica indica* Hardwicke

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Abstract. The Indian gerbil, *Tatera indica indica* Hardwicke, bathes by alternately rubbing its flanks (side-rubs) in the substrate, such as sand. Bathing frequencies are ordinarily low; but significant increments follow modifications made in pelage condition. When these are asymmetrical, more actions are directed towards the treated than the untreated side. Evidently, peripheral input regulates the behaviour.

Keywords. Sand bathing; side rubs; ventrum drag; coat care; *T. indica indica*.

1. Introduction

Most rodents possess an integrated system of behaviour for coat care (Griswold *et al* 1977). This includes washing of face, grooming and scratching (Barnett 1975). It is, however, observed that a number of species, particularly heteromyids and gerbils, bathe in substrates such as sand or dust to dress their fur (Eibl-Eibesfeldt 1951; Eisenberg 1963).

Sand-bathing behaviour of Indian gerbil, *Tatera indica indica* Hardwicke has however, not been studied. Results of experiments designed to study it are discussed here.

2. Material and methods

Adult gerbils trapped from fallow lands around Aligarh City were housed separately in wire-mesh enclosures (1.32×1×0.32 m). Wooden nest-boxes with paper strips were supplied for nesting; sand in dissection trays was given for bathing. The gerbils were fed on a mixed diet of cereals and vegetables; water was given *ad lib*. The experimental subjects were released in an all-glass aquarium (0.9×0.45×0.35 m) with a 10 cm layer of sand for substrate. The substrate was changed after each test. The aquarium was placed in a room fitted with 60W red bulbs for lighting. The observer sat at a distance of 2 m from the glass-front.

2.1 Experimental procedure

As the gerbils are nocturnal, all tests were made early in the activity period (1800—1930 hrs). Subjects were observed for 10 min intervals; actions were counted manually and timed by stop-watch.

The subjects were included at random in bisexual groups. Except for one group (controls 20 gerbils), gerbils of the other groups were tested after one of the following pre-treatments.

- (a) *Deprivation treatment*: The sand-trays were removed for a week and the gerbils were individually studied.
- (b) *"Wetting" of pelage*: Gerbils selected for this treatment were showered with water and observed in the test arena.
- (c) *Application of oil to pelage (symmetrical modifications)*: Symmetrical changes in pelage condition were made by applying 10 drops of lubricating oil to both the right and left sides of each subject.
- (d) *Application of oil to pelage (asymmetrical modifications)*: Oil drops were again used to alter the pelage on (i) right side only, (ii) left side, (iii) back and (iv) ventrum of gerbils belonging to four separate groups.

The results were statistically analyzed according to methods described by Bailey (1959) and Lehner (1979).

3. Results

The results are presented in table 1.

3.1 Description of movements

As observed, the sand-bathing behaviour of *T. indica* consisted of 'side-rubs'. It included well-coordinated movements or alternate extension and flexion of the body. These occurred rapidly as the gerbil shifted from one side to other. The two sides were thus attended alternately.

Another component identified was 'ventrum-rub' often displayed by male gerbils. It involved rapid extension and flexion of the body in a 'stretching' position. Lowering of the body on the substrate and dragging it in a forward direction, occurred separately from ventrum-rub. This did not involve any stereotype actions. Rollings on back were not seen.

3.2 Bathing frequencies

The untreated subjects released into the test-arena, were observed to bathe between bouts of exploratory activity. The number of actions was, however, limited to two at the most, in a 10 min observation period.

3.3 Relative frequencies after deprivation

The gerbils denied access to sand for a week displayed relatively more bathing by side-rubs than controls (Wilcoxon test; $P < 0.05$). The scores of actions were significantly higher for males than females ($\chi^2 = 12.5$; $P < 0.05$).

The number of actions directed towards the two sides were equal (Right rubs = $5.00 \pm \text{SE } 0.33$; Left rubs = $5.00 \pm \text{SE } 0.33$; table 1). Rubs were, however, made at irregular intervals; there was much exploration, but very little sand-digging.

3.4 Effect of "wetting"

After 'wetting' of pelage, the gerbils bathed intensely by side-rubbing. The increment in the number of actions as compared to bathing frequencies in

Table 1. The number of rubs performed by gerbils on each flank after various treatments given.

Expt. No.	Description of Subjects — sex, body wt (g)	Treatment	No. of actions: (Mean $\pm$ SE)	
			Right rub	Left rub
1	3 M (150, 157, 158)	Deprivation	5.0 $\pm$ 0.33	5.0 $\pm$ 0.33
	3 F (103, 130, 140)			
	2 M (152, 160)	Control	1.0	1.0
	2 F (121, 131)			
2	3 M (134, 148, 152)	Wetting	19.66 $\pm$ 4.48	18.33 $\pm$ 4.18
	3 F (125, 134, 142)			
	2 M (141, 171)	Control	2.0	2.0
	2 F (123, 141)			
3	2 M (124, 165)	Symmetrical modification by oiling	12.0 $\pm$ 0.707	11.25 $\pm$ 1.118
	2 F (125, 152)			
	3 M (141, 131, 149)	Control	1.0	1.0
	1 F (171)			
4	2 M (148, 168)	Asymmetrical modifications by oiling		
	1 F (132)	(a) Left side	3.00 $\pm$ 1.411	20.66 $\pm$ 0.59
	1 M (152)	(b) Right side	21.33 $\pm$ 1.23	2.66 $\pm$ 1.7
	2 F (131, 138)			
	1 M (132)	(c) Back	13.25 $\pm$ 0.87	14.25 $\pm$ 1.167
	1 F (132)			
	1 M (172)	(d) Ventrum	3.0	3.0
	1 F (137)			
		Ventrum drag		3.30 $\pm$ 1.30
	2 M (108, 138)	Control	2.0	2.0
	2 F (101, 103)			
		Ventrum drag		0.0

't' comparisons between treated and controls were found significant ($P < 0.05$) in each case.

M = Male; F = female.

untreated subjects, was obviously significant (Wilcoxon test; $P < 0.05$; table 1).

However, the same side was often rubbed in a sequence of 2-4 actions before attention was turned to the opposite flank. Thus the number of actions recorded on the two sides, was not always equal (left rubs = 19.66 $\pm$ SE 4.48; right rubs = 18.33 $\pm$ SE 4.18; table 1). The differences were not significant.

Exploratory activity and sand-digging were observed very late.

3.5 Effect of symmetrical modifications in pelage condition

Symmetrical modifications made in pelage condition by application of oil resulted in a large number of actions directed alternately towards the two flanks (table 1). The increments in bathing frequencies was significant (Wilcoxon test; $P < 0.05$).

Rubbings were, however, performed slowly; each act appeared deliberate, unlike the rapid actions noticed after wetting. The behaviour was eventually

interrupted by sand-digging. Exploration was seen only by the end of the observation schedule.

3.6 Behaviour after asymmetrical modifications

(i) Application of oil to only one flank, induced similarly significant increase in bathing frequencies (Wilcoxon test; $P < 0.05$; table 1). However, most actions were directed towards the treated side (left rubs = $20.66 \pm \text{SE } 0.59$; right rubs = $3.00 \pm \text{SE } 1.411$ or right rubs = $21.33 \pm \text{SE } 1.23$; left rubs = $2.66 \pm \text{SE } 1.7$; table 1) and differences between the number of actions recorded on treated and untreated flank were not only unequal, but significant (' t ' test; $P < 0.05$).

(ii) Oiling of back produced an equal number of side-rubs on the two flanks (left rubs = $13.25 \pm \text{SE } 0.87$; right rubs = $14.25 \pm \text{SE } 1.67$; table 1). Some subjects attempted to turn on their back, but without much success.

(iii) Subjects treated with oil on the ventrum displayed, however, 'dragging of ventrum' on substrate; though some side-rubs were also seen (table 1). The difference between frequencies of two components was significant (Wilcoxon test; $P < 0.05$); but no sequential relationship was evident. There was much sand-digging between ventrum drags.

4. Discussion

4.1 Bathing patterns in *T. indica*

Sand or dust-bathing by rodents consists of three main components (i) ventrum-rub, (ii) side-rub and (iii) rolls on back. These involve an equal number of fundamental acts, which are universal motor patterns in vertebrates (Eisenberg 1963). Evidently, it is by integrating a few basic acts that natural selection has produced functional bathing patterns. Side-rubs are, however, used to the exclusion of both 'ventrum-rub' and 'rolls on back' by *Gerbillus nanus* (Kirchshofer 1958). Thus, the bathing behaviour of *T. indica* is similar to that of *G. nanus*. If only side-rubs are performed, the absence of 'ventral glands' is indicated. These are residual; corresponding actions to clean ventrum are, therefore, not required. However, stereotyped rubbings of ventrum are often displayed by male *T. indica* because only males possess ventral scent glands (Prakash and Kumari 1979).

4.2 Functional relationships

Sand-bathing behaviour is prominently related to cleaning of fur (Borchelt *et al* 1976). This amounts to removal by rubbing in substrate of sebaceous secretions, mostly lipids, coming on to pelage; and similarly the materials that may get stuck or are applied to it (Borchelt *et al* 1976; Griswold *et al* 1977). If allowed to accumulate lipids are likely to mat the fur; bathing obviously restores pelage condition.

Seemingly sebaceous secretions that come on to the pelage in *T. indica*, have a particular spatial distribution. These are mostly extruded on flanks, and the area requires frequent rubbings to remove them. Deprivation thus enhances side-rub frequencies with the actions on both sides being equal (table 1). The same actions

are elicited by modifications made in pelage on flanks; with more actions directed on the 'treated' side if these are asymmetrical (table 1). As in other areas, pelage is cleaned without similar stereotyped actions.

Thus, bathing patterns of *T. indica* closely correspond to functional needs, as contended by Borchelt *et al* (1976).

4.3 Changes in temporal organization of bathing bouts

In subjects given access to sand, and tested without pretreatment, bathing action frequencies are ordinarily very low. Rubbings, if any, along with grooming and scratching, are seen between various activities as exploration, but without any time-sequence relationship with other events.

However, modifications in pelage condition as a result of pretreatments bring about a marked change in temporal organization of bathing bouts. Actions tend to 'cluster' at the beginning of observational periods, while other activities are relegated towards the end.

In spite of individual variations, temporal organization of bathing can thus be readily altered to suit requirement, as observed in kangaroo rats (Griswold *et al* 1977).

4.4 Breakdown of sequential patterning of bathing actions

While bathing, the gerbil, *T. indica* attends to its flanks alternately; in a single bout one action is thus directed to each side.

Such sequential patterning of action seems to breakdown, however, with asymmetrical manipulations of pelage condition. More actions are then directed towards the treated than the untreated side (table 1). In case of wetting, unequal distribution of water on the two sides may give a similar result (table 1).

Thus the sequential patterning of bathing actions is also rapidly altered according to need.

4.5 Regulation of behaviour

As observed earlier in kangaroo rats (Griswold *et al* 1977) and *Gerbillus nanus indus* (Prakash and Jain 1971) the responses of *T. indica* to changes in pelage condition also clearly suggest that 'peripheral input' mainly elicits the behaviour. Thus, the responses are quantitatively different with symmetrical and asymmetrical modifications made in the pelage condition (table 1).

Likewise, it appears that the behaviour is elicited from a central neural mechanism. It mediates the input to release bathing actions, and allows rapid adjustments thus in temporal and sequential patterning of actions, which are otherwise rigorously controlled (Griswold *et al* 1977).

Although peripheral-input 'threshold' in gerbils are not recognizable, even a small increase in input activates the mechanism. Slight wetting for example releases the behaviour in gerbils.

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Influence of spatial heterogeneity and prey density on predatory behaviour of the tropical spiders *Cyrtophora cicatrosa* (Stoliczka) and *Marpissa calcutaensis* (Tikader) (Araneae, Araneidae)

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Abstract. Tropical spiders *C. cicatrosa* and *M. calcutaensis* were allowed to predate individually in different terraria at different prey densities. Both the species were satiated within 5 hr of predation in all the volumes. Prey density exerted significant influence on the predatory behaviour of both the species. The predation increased with increasing prey density, but the volume of terraria exerted only marginal influence. The functional response curve of *C. cicatrosa* and *M. calcutaensis* was neither linear nor sigmoid, but was curvilinear representing type II response of Holling's model.

Keywords. Predatory behaviour; prey density; *Cyrtophora cicatrosa*; *Marpissa calcutaensis*.

1. Introduction

Spiders are known to achieve phenomenal densities in natural situations and consume astronomical quantities of insects; but these alone are not sufficient to demonstrate a significant role for them in the regulation of insect population. Rather they must be shown, by field observation or experimentation, to destroy prey in a density-dependent fashion (Greenstone 1978). They may be important to the balance of nature, but considerable quantitative evidence is lacking. Although a few publications are available on temperate spiders (Kajak 1965; Dabrowska-Prot *et al* 1966; 1968; Kajak *et al* 1968; Sandness and McMurtry 1970; Hardman and Turnbull 1974), the present study was undertaken to investigate the influence of spatial heterogeneity and prey density on the predatory behaviour of tropical spiders *Cyrtophora cicatrosa* and *Marpissa calcutaensis*.

2. Material and methods

C. cicatrosa, an orb-weaving spider with much evolved spinnerts, generally longer legs with specialised claws and silk glands (Palanichamy 1980) and *M. calcutaensis*, a hunting spider which does not spin its webs with small spinnerts, strong and stout legs, were collected from nearby fences of *Euphorbia antiquorum* at Palni (10°23'N 77°31' E). They were acclimated to laboratory conditions (30±1°C; RH 60-80%) and fed on mosquito, *Culex fatigans* for seven days. *C. cicatrosa* and *M. calcutaensis* feed mainly on dipterans, hymenopterans, hemipterans and orthopterans in

the field. *C. fatigans* which could be easily cultured in the laboratory were used as prey organisms in this work. Adult females were used and food was deprived to them for 24 hr before the experiments began. *Culex fatigans* served as prey, were cultured from the larval stages in the laboratory and only the adults which emerged during the day were used for feeding and experimental spiders (Palanichamy 1980).

Thirty individuals of each species were divided into six sets, each comprising five individuals. They were reared individually in transparent terraria of different volumes such as 25, 50, 100, 200 and 1000 mm³ and supplied with five adult *C. fatigans* per terrarium; the corresponding prey density was 0.2, 0.1, 0.05, 0.025, 0.01 and 0.005 fly/mm³, respectively. Similarly five series of experiments were conducted on prey density using 10, 20, 50, 100 and 200 *C. fatigans*/terrarium. The animals were released into the terraria containing a known number of test prey and the number of prey captured was replaced as and when they were captured to maintain a constant prey density.

Those mosquitoes which were chewed into food balls were counted as 'killed', chewed remains of prey were counted as 'consumed' and prey dying from other causes noted as 'dead'. Dead ones and spider silk were removed daily with a camel hair brush.

3. Results

3.1 Satiation

An animal is said to be satiated when it no longer accepts the offered prey after a period of active predation and feeding. The time from the start of feeding to voluntary cessation is the satiation time (Brett 1971). Satiation time was about 5 hr in the tested spiders irrespective of the volume of terraria. The number of

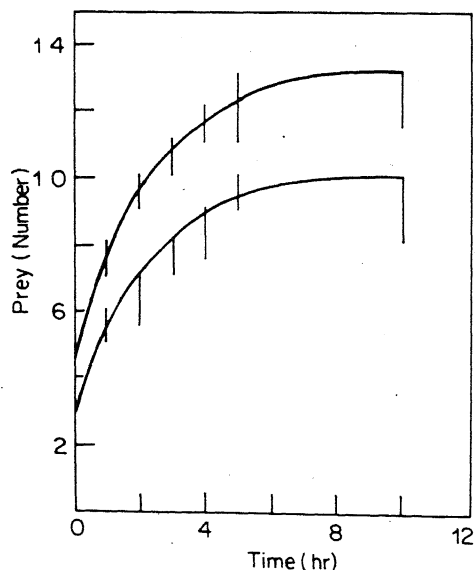


Figure 1. Predation of *C. cicatrosa* and *M. calcuttaensis* at 500 mm³ terrarium at the density of 20 flies/terrarium as a function of time.

C. fatigans predated as a function of successive periods of *C. cicatrosa* and *M. calcuttaensis* when allowed to predate in the terrarium volume of 500 mm³ is shown in figure 1. About 50% of the prey were predated within the first 1 hr and the predation decreased during subsequent 1 hr intervals.

Table 1. Mosquito predation by *C. cicatrosa* and *M. calcuttaensis* in relation to prey density at different terraria volume.

Volume of Terraria (mm ²)	Prey density (No.)	<i>C. cicatrosa</i>		<i>M. calcuttaensis</i>	
		Prey killed (No.)	Prey consumed (No.)	Prey killed (No.)	Prey consumed (No.)
25	5	3.2 ± 0.2	2.3 ± 0.2	3.5 ± 0.3	3.3 ± 0.2
	10	6.2 ± 0.4	4.8 ± 0.3	7.8 ± 0.5	4.9 ± 0.4
	20	9.1 ± 0.5	6.6 ± 0.4	9.7 ± 0.8	6.6 ± 0.5
	50	14.2 ± 0.9	9.6 ± 0.7	15.2 ± 1.1	10.1 ± 0.7
	100	18.0 ± 1.3	11.9 ± 0.9	18.6 ± 1.4	12.6 ± 1.1
	200	—	—	—	—
50	5	4.2 ± 0.4	2.6 ± 0.2	4.0 ± 0.3	3.9 ± 0.3
	10	6.7 ± 0.5	5.1 ± 0.3	8.1 ± 0.5	5.4 ± 0.4
	20	9.3 ± 0.7	6.9 ± 0.4	10.1 ± 0.7	6.9 ± 0.7
	50	14.7 ± 1.2	9.7 ± 0.7	15.6 ± 1.2	10.5 ± 0.9
	100	18.4 ± 1.4	12.4 ± 1.0	19.0 ± 1.4	12.7 ± 1.1
	200	—	—	—	—
100	5	4.6 ± 0.3	2.9 ± 0.2	4.8 ± 0.4	4.0 ± 0.3
	10	7.1 ± 0.5	5.6 ± 0.4	8.5 ± 0.6	5.6 ± 0.5
	20	10.1 ± 0.7	7.1 ± 0.5	10.7 ± 0.8	7.2 ± 0.6
	50	14.9 ± 1.0	10.9 ± 0.7	16.0 ± 1.4	10.9 ± 1.0
	100	18.9 ± 1.2	10.5 ± 0.8	19.6 ± 1.6	13.2 ± 1.1
	200	19.4 ± 1.4	12.8 ± 0.9	19.9 ± 1.5	13.4 ± 1.2
200	5	4.8 ± 0.3	3.1 ± 0.2	5.0 ± 0.4	4.1 ± 0.2
	10	7.5 ± 0.5	5.8 ± 0.3	8.9 ± 0.7	5.9 ± 0.4
	20	10.5 ± 0.6	7.6 ± 0.4	11.3 ± 0.9	7.6 ± 0.6
	50	15.4 ± 0.9	10.5 ± 0.6	16.4 ± 1.2	11.0 ± 0.8
	100	19.2 ± 1.1	12.8 ± 0.9	19.7 ± 1.4	13.4 ± 1.0
	200	19.7 ± 1.3	13.1 ± 1.0	20.4 ± 1.4	13.7 ± 1.1
500	5	5.0 ± 0.3	3.3 ± 0.2	5.0 ± 0.3	4.3 ± 0.3
	10	7.7 ± 0.5	6.1 ± 0.4	9.2 ± 0.5	6.4 ± 0.3
	20	10.9 ± 0.7	8.3 ± 0.6	11.5 ± 0.8	8.0 ± 0.4
	50	15.7 ± 1.2	10.9 ± 1.0	16.7 ± 1.1	11.2 ± 0.8
	100	19.7 ± 1.4	13.1 ± 1.0	20.0 ± 1.3	13.7 ± 0.9
	200	20.1 ± 1.6	13.8 ± 1.4	21.0 ± 1.6	14.1 ± 1.2
1000	5	5.0 ± 0.4	3.9 ± 0.3	5.0 ± 0.4	4.7 ± 0.4
	10	8.1 ± 0.5	6.3 ± 0.4	9.7 ± 0.7	6.9 ± 0.5
	20	11.0 ± 0.8	8.7 ± 0.6	11.9 ± 0.8	8.4 ± 0.5
	50	16.1 ± 1.1	11.5 ± 0.7	17.1 ± 1.2	11.5 ± 0.9
	100	19.7 ± 1.2	13.4 ± 0.9	20.4 ± 1.4	13.9 ± 1.0
	200	20.3 ± 1.4	14.1 ± 1.1	21.6 ± 1.7	14.9 ± 1.2

Each value represents the mean (±S.D) of 5 individuals.

Table 2. Mosquito predation by *C. cicatrosa* and *M. calcutaensis*; rate of predation as a function of prey density and volume of terraria.

Density of prey (No.)	Volume of Terraria (mm ³)					
	25	50	100	200	500	1000
<i>C. cicatrosa</i>						
5	0.18	0.24	0.27	0.28	0.29	0.29
10	0.36	0.39	0.41	0.44	0.43	0.47
20	0.55	0.54	0.59	0.61	0.64	0.65
50	0.83	0.86	0.87	0.90	0.92	0.94
100	1.05	1.08	1.11	1.12	1.14	1.15
200	—	—	1.14	1.15	1.18	1.19
<i>M. calcutaensis</i>						
5	0.20	0.27	0.28	0.29	0.29	0.29
10	0.45	0.47	0.50	0.52	0.54	0.57
20	0.57	0.59	0.63	0.66	0.68	0.70
50	0.89	0.91	0.94	0.96	0.98	1.00
100	1.09	1.10	1.44	1.15	1.17	1.20
200	—	—	1.17	1.20	1.23	1.27

3.2 Predation as a function of spatial heterogeneity

The volume of terraria exerted only marginal influence on the predatory behaviour of *C. cicatrosa* and *M. calcutaensis*. For instance, *C. cicatrosa* killed 6.2 and 8.1 flies at a density of 10 flies/terrarium in 25 and 1000 mm³, respectively (table 1). Corresponding values for *M. calcutaensis* were 7.8 and 9.7 flies.

The number of prey successfully captured can also be considered as the rate of successful attack (predation) which can be calculated considering the exposure time of the predation and the number of prey captured. The rate of predation for the predator-prey interactions, i.e. *C. cicatrosa* vs *C. fatigans* and *M. calcutaensis* vs *C. fatigans* were 0.36 and 0.45 at the terrarium volume of 25 mm³ increased to 0.47 and 0.57 at the terrarium volume of 1000 mm³. The same trend persisted at the tested densities of 10, 20, 50, 100 and 200 flies/terrarium also (table 2). Terrarium size has no influence on the rate of successful attack.

3.3 Predation as a function of prey density

The number of prey killed as a function of prey density can be expressed in terms of prey risk, i.e. the number of prey killed per unit time per unit density. The prey risk increased with increasing prey density. *C. cicatrosa* and *M. calcutaensis* have increased prey capture with increasing density of prey up to 100 prey per terrarium and subsequently levelled off (table 1). Rate of attack increased with increasing prey density irrespective of the predator (figure 2).

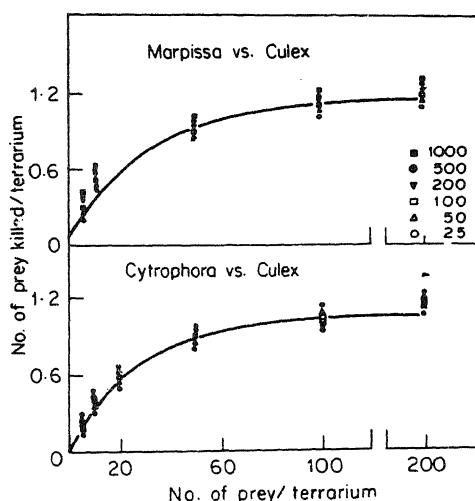


Figure 2. Rate of predation (*M. calcutaensis* vs *C. fatigans* and *C. cicatrosa* vs *C. fatigans*) as a function of prey density.

4. Discussion

There are several reports on the satiation time of fish (e.g. Brett 1971) and insects (e.g. Pandian *et al* 1978). But there is paucity of information about the satiation time of spiders. The present work showed that *C. cicatrosa* and *M. calcutaensis* required 5 hr for satiation which is much longer when compared to the fish *Onchorhynchus nerka* that satiated by 1 hr (Brett 1971); the dragonfly nymph *Mesogomphus lineatus* that took only 40 min (Pandian *et al* 1978). The greater lapse in the satiation time of spiders may be due to their mode of feeding. They cannot ingest the prey as such, as in other predators, but the process involves (i) handling the prey, (ii) dissolving prey tissues externally with digestive enzymes introduced into wounds made by the fangs, (iii) ingestion of semidigested broth and (iv) discarding of the remains (Palanichamy 1980). Hence, they require a longer time for satiation when compared to that of other predators which ingest the prey outright.

Majority of studies on the predatory behaviour have been made with predators and prey kept in relatively simple containers (Chant and Turnbull 1966; Dabrowaka-Prot *et al* 1966; 1968; Kajak 1965; Kajak *et al* 1968; Sandness and McMurtry 1970). However the predatory behaviour in response to prey density may be altered in a spatially complex environment (Griffiths 1969; Huffaker *et al* 1963; Levins 1966; MacArthur and Pianka 1966). But in the present investigation, the volume of different terraria exerted marginal influence on the predatory behaviour of the spiders *C. cicatrosa* and *M. calcutaensis*.

It is known for a variety of predators that a predator attacks a greater number of prey as the prey density increases. Solomon (1949) has called this as functional response which is of three types (Holling 1963). Type I is a linear rise in the consumption of prey to a plateau characteristic of filter feeder. Type II curve is a negative accelerated rise to a plateau and is representative of most invertebrates

and some fish. The type III functional response is a sigmoid curve rising to a plateau which is characteristic of vertebrates. Spiders do respond functionally to prey availability. In spite of their functional response, there is species difference in the predatory behaviour *i.e.*, the jumping spider *M. calcutaensis* killed more number of prey when compared to that of weaving spider *C. cicatrosa* (figure 1). Hunting spiders may be able to change their rate of attack than do the web building spiders which are filter feeders and generally less mobile than the hunting spiders (Pointing 1966; Palanichamy 1980).

The functional response is said to be non-regulating in some species of spiders (Kiritani *et al* 1972). In wolf spider *Pardosa vancouveri*, type III response is seen (Hardmann and Turnbull 1974). In the present investigation the functional response of both the species of spiders *C. cicatrosa* and *M. calcutaensis* is curvilinear representing the typical invertebrate type of functional response *i.e.*, Type II response of Holling (1965). Nonlinearity may be due to the following reasons: Though the time available to the predator for hunting prey decreases as the rate of prey capture increases, an individual predator cannot consume food above a satiation rate of prey exploitation, at which point the functional response levels off.

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Population dynamics and energy budgets of *T. pulvinata* Uvarov and *P. infumata* Brunn (Orthoptera: Acrididae)

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Abstract. The population dynamics and energy budgets of two grasshopper species *Tristria pulvinata* Uvarov and *Phlaeoba infumata* Brunn were studied in a grassland at Kurukshetra. Males outnumber females. The aboveground net primary production was more in stand I as compared to that in stand II. Mean secondary production was 753.88 mg m⁻². Field population of the grasshopper species consumed 308.57 K cal m⁻² during the study period.

Keywords. *T. pulvinata*; *P. infumata*; secondary production; energy budgets.

1. Introduction

Secondary producers interact within the ecosystem as consumers, utilizing and recycling the products of net secondary production. Numerically, arthropods generally dominate the animal populations of natural ecosystems. Only recently have ecologists begun to consider the functional role of the arthropod community within the terrestrial ecosystems. Considerable work on related fields has been done in various other grassland ecosystems (Smalley 1960; Odum *et al* 1962; Gyllenberg 1969; Nakamura *et al* 1971; Bhatnagar and Pfadt 1973; Delvi and Pandian 1979; Vats and Kaushal 1981). To clarify the role of consumers of *Desmostachya bipinnata* dominated grassland, the population density and productivity of two grasshopper species were estimated for 2 yrs from June 1976 to May 1978 in the present investigation.

2. Material and methods

2.1 The study area

The study site is situated at the university campus at Kurukshetra (29° 58' N and 76° 51' E). The climate of Kurukshetra is monsoonic, with an average rainfall of 800 mm. During the study period, the mean maximum temperature varied from 21.3°C (January) to 37.8°C (June) while the mean minimum temperature ranged from 6°C (December) to 24.6°C (June). There are three wet months of rainy season (July to September) and nine dry months. The dry period is further divisible into a cool dry period from October to February (winter season) and a hot dry period from March to June (summer season). The seasonal data on temperature and rainfall of the study site are presented elsewhere (Vats and Kaushal 1981).

2.2 Vegetation

Two types of vegetation were recognized. An area with mixed-grasses was designated as stand I, whereas an area dominated by the perennial grass *Desmostachya bipinnata* was referred to as stand II.

2.3 Estimation of population density, biomass, secondary productivity and energy budget

Population density was studied using removal-trapping method since the insects once trapped had the least chance to escape. For this purpose, a trap of 1.75 m² with a small entrance (82 × 85 cm) on one side, 40 cm above the ground level was constructed. Wire gauge of 5 meshes per cm was fixed on the sides and top of the trap. It was carried from one sampling spot to another with handles fixed on the opposite sides. The trap was lowered randomly to the ground at ten different spots, five per stand. While moving the trap, the tip of the grassblades was not disturbed. The insects thus entrapped were collected, oven-dried at 60°C, and weighed in a single pan electric balance (accuracy up to 0.1 mg).

Secondary production was calculated by using sampling data to compute the mean biomass present during a sampling period. Production was calculated as:

$$P = S + \sum_{i=2}^n \frac{1}{2}(N_i + N_{i-1}) \cdot (W_i - W_{i-1})$$

where N_i = the number of grasshoppers present at time i ; W_i = the mean weight per grasshopper at i ; i = the sample time; S = the standing crop at time $i = 1$. It was assumed that $N_i \leq N_{i-1}$ and $W_i \geq W_{i-1}$. When W_i was less than W_{i-1} , the production was considered as zero. This expression was also used by others (Wiegert 1965; van Hook 1971 and Riegert and Varley 1973).

The energy content of grasshoppers, plant material and faecal matter was determined by using an adiabatic bomb calorimeter (Phillipson 1964). All the materials were dried in an oven at 60°C, ground to a fine powder by an electric grinder and made into pellets.

For studies on feeding, grasshoppers brought from the field and confined to small cages were fed for 24 hr with fresh plant material of *Desmostachya bipinnata* kept in small tubes containing water. At the end of the experiment, the grasshoppers were weighed; unconsumed plants and egesta were collected and oven-dried at 80°C till a constant weight was obtained. The dry weight equivalents of grasshoppers and plant material were obtained by oven-drying of these materials. Food consumption was calculated as the difference between initial weight of the food provided and the unconsumed material at the end of the experiment after correcting for the weight loss in the food material due to respiration and transpiration. Approximate digestibility (AD) was calculated using the expression:

$$AD = \text{Assimilation/consumption} \times 100$$

* Seasonal mean biomass for the estimation of annual consumption was determined by using Petruszewicz and Macfadyen (1970) expression.

$$B = 1/K \sum_{i=1}^{i=k} B_i$$

where, B_i is the successive standing crop estimated and K is the number of sampling times.

3. Results

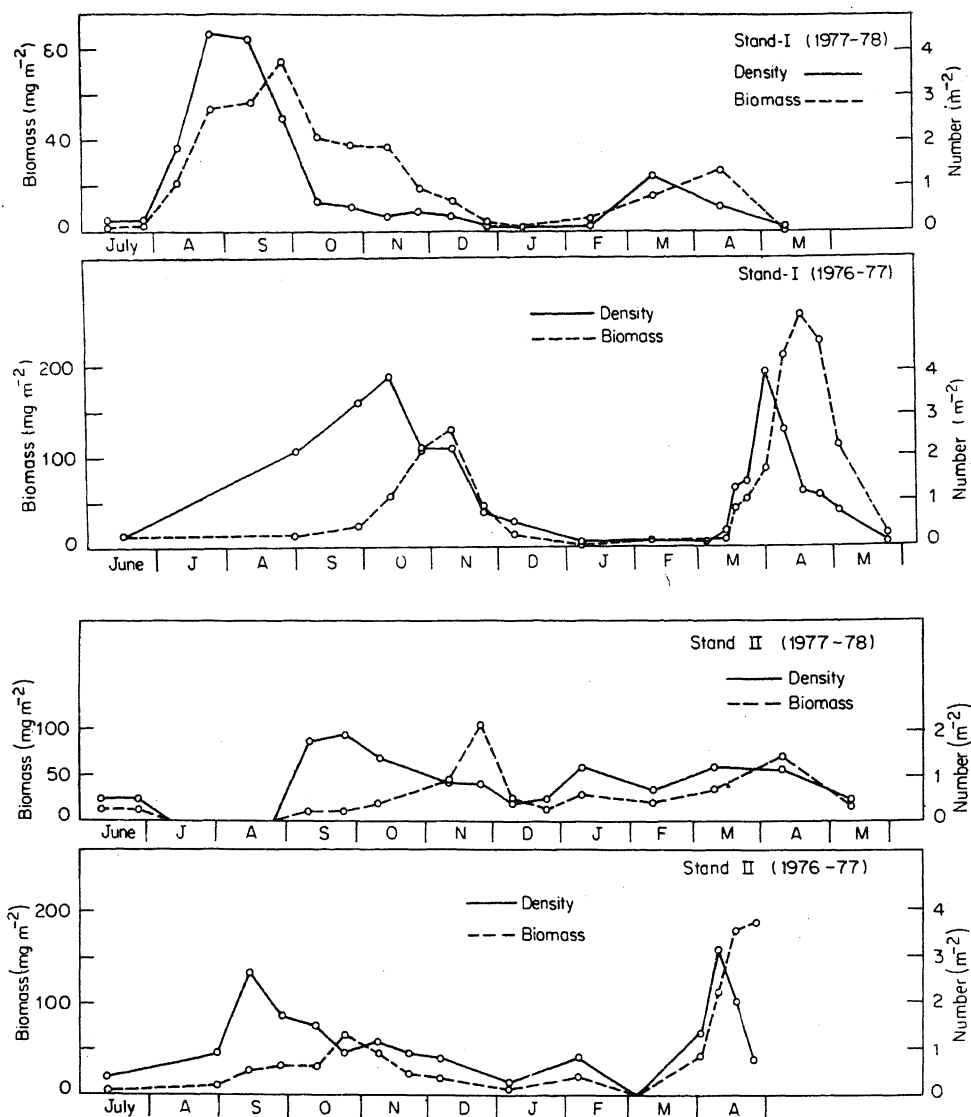
3.1 Producers

Eighty-eight plant species were observed in both the stands. Of these, 22 were confined to stand I and 9 to stand II; the remaining were common to both the stands. The maximum number of species was recorded in rainy season (69 in stand I and 58 in stand II) and the minimum in hot dry months of summer (17 in stand I and 13 in stand II). In winter, there were 35 and 30 species in stands I and II, respectively. The live-shoot biomass varied from 1.7 ± 1 (January) to 1487.8 ± 4.8 g m⁻² (September) in stand I and from 7.8 ± 3.6 (January) to 737.6 ± 2.5 g m⁻² (October) in stand II. The total live shoot biomass in rainy, winter and summer seasons was 2351.1, 428.8 and 835.5 g m⁻² in stand I and 986.8, 1013.1 and 502.7 g m⁻² in stand II, respectively. Aboveground net primary production was 2307 g m⁻² yr⁻¹ on stand I and 1208.4 g m⁻² yr⁻¹ on stand II.

3.2 Population density and biomass

Variations in the population densities and biomass of grasshoppers in relation to the seasons are shown in figures 1-4. Two peaks of population density (adult + nymphs) were obtained on both the stands; one during rainy season (first emergence) and another at the beginning of the summer season (second emergence). Hence two generations of both the species were recorded in a year. Population density in the first year reached its maximum value towards the end of rainy season *i.e.* in October on stand I and in September on stand II. Thereafter, it declined with the approach of winter season till February. Again emergence of nymphs occurred, hence second peak was obtained on both the stands during the beginning of summer season *i.e.* March and April. Density declined thereafter because of extreme summer on both the stands. In the two grasshopper species, the average value of adults was 6.3% with 22.8% biomass and nymphs 93.7% with 77.2% biomass.

Grasshoppers entering the population in early summer (March and April) and early rainy season consisted of small nymphs, resulting in sharp decrease in the biomass. Two peaks of biomass were obtained on both the stands, *i.e.* one towards the end of April and another in the beginning of winter period as nymphs of latter stages and adults were present. Biomass values decreased considerably towards extreme summer and winter seasons because of low population density.



Figures 1-4. Variation in population density and biomass of grasshoppers.

3.3 Secondary production

Data on seasonal changes in the mean dry weight per individual and secondary production on both the stands are given in tables 1 and 2. However, growth rates are generally not constant because of the presence of different instars of both the species of grasshoppers which hatch at different intervals. The production estimates do not include exoskeleton material moulted by the growing nymphs as it was not possible to identify it in the field and also because of the presence of other insect fauna. The initial standing crop (mg m^{-2}) was used as a minimal estimate of production up to the time, sampling was started.

Table 1. Mean weight per individual and secondary production of grasshoppers on stand I during June 1976 to May 1978.

Date		Mean weight (m ⁻²)	Secondary production* (mg m ⁻²)
1976	15/6	41.18	(9.06)
			Total 9.06
	31/8	6.20	(12.71)
	28/9	7.25	2.74
	12/10	14.71	2.59
	26/10	49.53	103.07
	9/11	59.72	22.06
	23/11	57.58	0.00
	7/12	24.43	0.00
1977	7/1	9.27	0.00
	7/2	38.36	3.20
			Total 146.42
	4/3	54.27	(5.97)
	12/3	27.45	0.00
	17/3	40.51	11.17
	22/3	44.34	5.46
	31/3	22.83	0.00
	8/4	84.20	196.38
	16/4	206.30	229.55
	24/4	202.28	0.00
	2/5	140.96	0.00
1978	27/5	125.64	0.00
			Total 448.53
	11/7	6.32	(1.39)
	27/7	9.87	0.80
	11/8	11.71	1.89
	26/8	12.64	2.86
	11/9	13.49	3.64
	26/9	33.46	64.70
	11/10	60.26	39.53
	26/10	67.95	4.81
	11/11	109.74	18.81
	26/11	41.53	0.00
1978	11/12	39.59	0.00
	26/12	24.55	0.00
	11/1	23.91	0.00
	11/2	55.45	3.47
			Total 141.90
	11/3	13.18	(16.47)
	11/4	67.49	47.88
	11/5	2.18	0.00
			Total 64.35

*New cohort for secondary production considered during March (first emergence) and July (second emergence) when eggs hatch.

Table 2. Mean weight per individual and secondary production of grasshoppers on stand II during June 1976 to May 1978.

Date	Mean weight (mg insect ⁻¹)	Secondary production* (mg m ⁻²)
1976		
12/7	9.68	(3.29)
30/8	10.46	0.49
13/9	9.87	0.00
27/9	17.81	17.23
11/10	20.74	4.69
25/10	72.46	62.06
8/11	39.41	0.00
22/11	25.36	0.00
6/12	23.02	0.00
1977		
6/1	28.13	2.62
6/2	29.01	0.45
	Total	90.83
1/4	28.39	(38.89)
9/4	34.82	14.66
17/4	87.42	137.81
25/4	241.20	218.37
10/6	22.93	0.00
26/6	23.20	0.12
	Total	409.85
10/9	4.22	(7.17)
25/9	4.90	1.20
10/10	12.64	12.27
10/11	53.64	44.08
25/11	131.73	62.08
10/12	70.82	0.00
25/12	24.61	0.00
1978		
10/1	24.77	0.13
10/2	26.73	0.46
	Total	127.39
10/3	30.82	(34.83)
10/4	61.58	34.61
10/5	35.98	0.00
	Total	69.44

*New cohort for secondary production considered during March (first emergence) and July (second emergence) when eggs hatch.

3.4 Energy content

Table 3 comprises energy values of different biological materials. The energy value for grasshoppers > faeces > plant material (see also Bailey and Riegert 1973; Campbell *et al* 1976).

3.5 Assimilation rates

The relationship between food consumption and egesta is presented in table 4. When calculated against duration of a stage per day, the adult females consumed more than the males of both species. The female also consumes more than male in *Parapleurus alliaceous* (Matsumoto 1971); in *Paprides nitidus*, *Siagus australis* and *Brachaspis nivalis* (White and Watson 1972); in *Poeciloceris pictus* (Delvi and Pandian 1979) and in *Parahieroglyphus bilineatus* (Vats and Kaushal 1981). The rate of egestion is higher in females than in males of both the species. Similar results were obtained by Matsumoto (1971) and Vats and Kaushal (1981).

Males were more efficient in assimilating the consumed food than females. Similar observations were obtained by White (1978); Vats and Kaushal (1981); but in *B. nivalis*, females have higher AD than males (White 1978).

Table 3. Energy values of biological materials.

Material	Energy value (J mg ⁻¹ dry wt.)	
<i>T. pulvinata</i> (all stages)	21.62	} Mean 21.445
<i>P. infumata</i> (all stages)	21.27	
Faeces (mixed)	20.4	
Plant material	19.56	

All values on oven dry weights.

Table 4. Relationship between food consumption and egestion for the two grasshopper species fed on *Desmostachya bipinnata*.

Stage		Initial biomass (J insect ⁻¹)	Consumption (J insect ⁻¹ day ⁻¹)	Egesta (J insect ⁻¹ day ⁻¹)	AD (%)
<i>T. pulvinata</i>					
Fifth instar	M	1189.25	287.12 (34.74)	179.91 (15.59)	37.34
	F	4108.35	1000.82 (51.66)	752.53 (60.11)	24.81
Adult	M	1599.98	278.35 (28.09)	188.89 (20.15)	32.14
	F	5081.46	942.97 (44.02)	759.21 (44.14)	19.49
					Mean 28.45
<i>P. infumata</i>					
Fifth instar	M	723.22	227.1 (21.28)	154.07 (12.29)	32.16
	F	610.68	762.77 (52.12)	592.68 (56.35)	22.29
Adult	M	1063.6	187.97 (18.43)	131.59 (13.12)	29.99
	F	3786.41	676.07 (35.62)	555.23 (49.16)	17.87
					Mean 25.58

Standard error given in parentheses.

Table 5. Consumption by the field population of *T. pulvinata* and *P. infumata* during June 1976 to May 1978.

Season	No. of days	*Mean biomass (mg m ⁻²)	Consumption (cal m ⁻²)
Stand I			
Summer (March-June)	244 ×	76.629×5.131**×0.935***	89700.86
Rainy (July-Sept.)	184 ×	12.617×5.131×0.935	11137.49
Winter (Oct.-Feb.)	302 ×	45.105×5.131×0.935	65349.95
			Total 166.19 kcal.m ⁻²
Stand II			
Summer	244 ×	62.927×5.131×0.935	73661.49
Rainy	184 ×	9.49 ×5.131×0.935	8377.17
Winter	302 ×	41.648×5.131×0.935	60341.31
			Total 142.38 kcal m ⁻²

*Mean biomass from tables 1 and 2; **Energy content; ***Weight specific consumption

3.6 Effect of field population on the vegetation

On the basis of weight-specific consumption, determined under laboratory conditions and the time series biomass determined in the field, the effect of field population on vegetation can be determined. The total annual consumption has been obtained by totalling the consumption for all seasons calculated as under:

$$C_s = B \times N \times C_w$$

where C_s is the seasonal consumption, B is the mean seasonal biomass, N is the number of days in a season during which the hoppers were available and C_w is the weight-specific consumption. Average weight-specific consumption for adults of both the species comes to 0.935 cal mg⁻¹ day⁻¹ (tables 3 and 4).

Total consumption by grasshoppers on both the stands was 308.57 kcal m⁻² during June 1976 to May 1978. Consumption was higher on stand I (166.19 kcal m⁻²) than on stand II (142.38 kcal m⁻²) because of higher population density (table 5).

4. Discussion

A maximum density of 4.34 m⁻² on stand I and 3.19 m⁻² on stand II as observed presently appear to be rather low because of our trapping technique as compared to the densities in other grassland communities (20 m⁻² in terrestrial ecosystem Parker 1952; 31.5 m⁻² of *Chorthippus parallelus* in *Agrotis tenuis* grassland Gyllenberg 1969; 8.5 m⁻² in *Andropogon virginicus* van Hook 1971; 4.4 m⁻² in Natural grassland Riergert *et al* 1974 and 3.76 m⁻² in short-grass prairie van Horn 1972).

Thus different species are dominant in different communities depending upon the vegetation and climatic conditions of the regions concerned.

Maximum biomass values were 257.87 mg m⁻² on stand I and 190.55 mg m⁻² on stand II. Biomass values vary with the type of vegetation (817.4 mg m⁻² in the old field and alfalfa field Wiegert 1965; 1150.32 mg m⁻² in the oldfield ecosystem at Tennessee van Hook 1971; 189 mg m⁻² in the mixed-grass forbs at Georgia Menhinick 1967 and 83.72 mg m⁻² in natural grassland Riegert *et al* 1974).

Maximum values of population density which normally occur in the beginning of rainy season, were recorded in October on stand I and in September on stand II in the first year of study period. This was due to temporary water-logging of the area under investigation. Emergence occurred in the rainy season on both the stands in the second year when both temperature and moisture were favourable.

Criddle (1917) reported that abundance of grasshoppers is negatively correlated to rainfall. Pradhan and Peswani (1961) stated that high and low temperature adversely affected the population density of *Hieroglyphus nigrorepletus*.

Minimum values of population density were obtained during extreme summer (May-June) and winter seasons (December-February) when productivity was low, rainfall scanty and temperature high in summer and low in winter. These factors are also known to affect the population density of grasshoppers (Andrewartha and Birch 1954; Clark *et al* 1967; Bhatnagar and Pfadt 1972; Dempster 1975).

Net secondary production of 810.26 mg m⁻² on stand I and 697.51 mg m⁻² on stand II in the present study appears to be higher as compared to the secondary production in other grasslands. Net secondary production of grasshoppers in an oldfield ecosystem at Michigan was 122 mg m⁻² in 1959; 71.7 mg m⁻² in 1960 and 878.9 mg m⁻² in alfalfa field in 1960 (Wiegert 1965). Riegert and Varley (1973) on the basis of collective biomass data obtained the values of 45.97, 24.51 and 47.29 mg m⁻² in the unstressed, burned and grazed plots respectively. The relatively high values reported in the present study were probably due to higher primary productivity or by greater accuracy of the equation used here.

Assimilation efficiency in the present study falls within 27-80% as reported by Odum and Smalley (1959), Wiegert and Evans (1967); Delvi and Pandian (1979) and White (1978).

Field population of grasshoppers ingested 0.9% of the net primary production indicating that they had little impact on the primary production. Wiegert (1965) also reported that total ingestion by grasshoppers was less than 0.5% in the oldfield and 2.5% on the alfalfa field.

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Population dynamics and biomass production of aboveground insects in a tropical grassland

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Abstract. Population dynamics and biomass of aboveground insects were studied in a tropical grassland. A total of 30 species belonging to five insect orders viz., Odonata, Mantoidea, Blattoidea, Neuroptera and Diptera were collected. Maximum population density and biomass values on stands I and II were determined.

Keywords. Aboveground insects; population density; biomass.

1. Introduction

Numerically, arthropods dominate the animal populations of natural ecosystems. There have been relatively few studies on the population density and biomass of total insect fauna both under temperate and tropical conditions. While majority of the studies are confined to the taxonomic composition of insect fauna (Ricou 1967; Evans and Murdoch 1968; Igarshi 1973; Riegert *et al* 1974; Janzen and Pond 1975; Lamotte 1975 and Vats and Singh 1978) attempts to evaluate population density and biomass of insecta as a whole are few (Ricou 1967; Riegert *et al* 1974; Lamotte 1975 and Vats and Singh 1978).

The present paper records the population density and biomass of five insect orders Odonata, Mantoidea, Blattoidea, Neuroptera and Diptera during period June 1976 to May 1978.

2. Materials and methods

Materials and methods have been discussed in our earlier paper.

3. Results and discussion

3.1 Species composition

Species composition was first considered (table 1). A total of 30 species belonging to 15 families of five orders were recorded. Of the 30 species, 2 were confined to stand I and 4 to stand II; whereas the remaining species were common to both the stands. On both the stands, the maximum number of individuals belonged to family Mantidae (table 1).

Species composition in different communities has been reported earlier. Ricou (1967) reported 44 species from sown plants at site La Breteque and 96 species from

Table 1. Species composition, number of individuals collected and their per cent contribution on two stands during June 1976 to May 1978.

Taxon	Number on stand I		Number on stand II	
	Total	%	Total	%
<i>Odonata</i>				
Coenagrionidae				
<i>Ceriagrion coromandelianum</i> F.	1	0.47	2	1.04
<i>Rhodischnura nursei</i> Morton	7	3.29	—	—
<i>Tetrathemis platyptera</i> Selys.	6	2.82	1	0.52
Agriidae				
<i>Ischnura senegalensis</i> Rambur	7	3.29	10	5.18
Libellulidae				
<i>Crocothemis servilia</i> Drury	13	6.10	4	2.07
<i>Pantala flavescens</i> Fabr.	3	1.41	2	1.04
<i>Orthetrum sabina</i> Drury	3	1.41	2	1.04
<i>Mantoidea</i>				
Mantidae				
<i>Didymocorypha lanceolata</i> Fabr.	13	6.10	13	6.73
<i>Ephestisula intermedia</i> Werner	3	1.41	1	0.52
<i>Humbertiella ceylondica</i> Sauss.	—	—	2	1.04
<i>Mantis religiosa</i> Linn.	25	11.73	33	17.10
<i>Schizocephalus bicornis</i> Linn.	8	3.75	7	3.63
<i>Blattoidea</i>				
Blattidae				
<i>Blatella humbertiana</i> Sauss.	6	2.82	4	2.07
<i>Mareta</i> sp.				
<i>Neuroptera</i>				
Hemerobiidae				
<i>Palpares paldus</i> Rbr.	1	0.47	—	—
<i>Suphalomitus verbosus</i> Walk.	4	1.88	1	0.52
Myrmeliontidae				
<i>Heliocoelus dicax</i>	2	0.94	5	2.59
<i>Diptera</i>				
Tabanidae				
<i>Tabanus</i> sp.	19	8.92	3	1.55
<i>Tabanus hiliaris</i> Walk.	6	2.82	4	2.07
Bombyliidae				
<i>Anthrax</i> sp.	—	—	1	0.52
Asilidae				
<i>Promachus</i> sp.	3	1.41	2	1.04
Calliphoridae				
<i>Chrysomia</i> sp.	3	1.41	10	5.18
<i>Lucilla</i> sp.	2	0.94	5	2.59
<i>Rhynchomyia</i> sp.	5	2.34	6	3.11
<i>Strongyloneura</i> sp.	6	2.82	4	2.07
Sarcophagidae				
<i>Sarcophaga</i> sp.	1	0.47	8	4.14
<i>S. albiceps</i> Meigen	—	—	2	1.04
Tachinidae				
Unidentified	47	22.06	30	15.54
Muscidae				
<i>Orthelia caesarion</i> Meig.	18	8.45	24	12.43
Trypetidae				
Unidentified	—	—	1	0.52

permanent pasture at site La Vielle in France during 1967-68; Evans and Murdoch (1968) recorded a total of 437 species (35 Odonata, 8 Neuroptera and 394 Diptera) in an alfalfa field over a period of 11 yrs during IBP studies at Kawatabi, Japan; Igarshi (1973) reported 17 species (1 Mantoidea, 1 Neuroptera and 15 Diptera); Pimental and Wheeler (1973) recorded 87 species (14 Odonata, 1 Mantoidea, 7 Neuroptera and 65 Diptera); Janzen and Pond (1975) reported 99 species (1 Neuroptera and 98 Diptera) at Bridford and 104 Dipterans at Michigan in 1972.

3.2 Population density and biomass

Data on the population density and biomass of insecta (sum total of all five orders) are plotted in figures 1-4. The minimum values were obtained either in summer (April-June) or in winter (October-February) and the maximum values during the rainy season (late June-September). Maximum biomass values were also obtained during rainy season on both the stands.

Seasonal variations in population density and biomass of all the insect orders were discussed earlier (Kaushal 1979). The maximum values of population density, biomass, families and species are given for each of the insect order. Table 2 shows the per cent contribution of different orders towards total insect population density and biomass and table 3 shows the per cent contribution of families within that order.

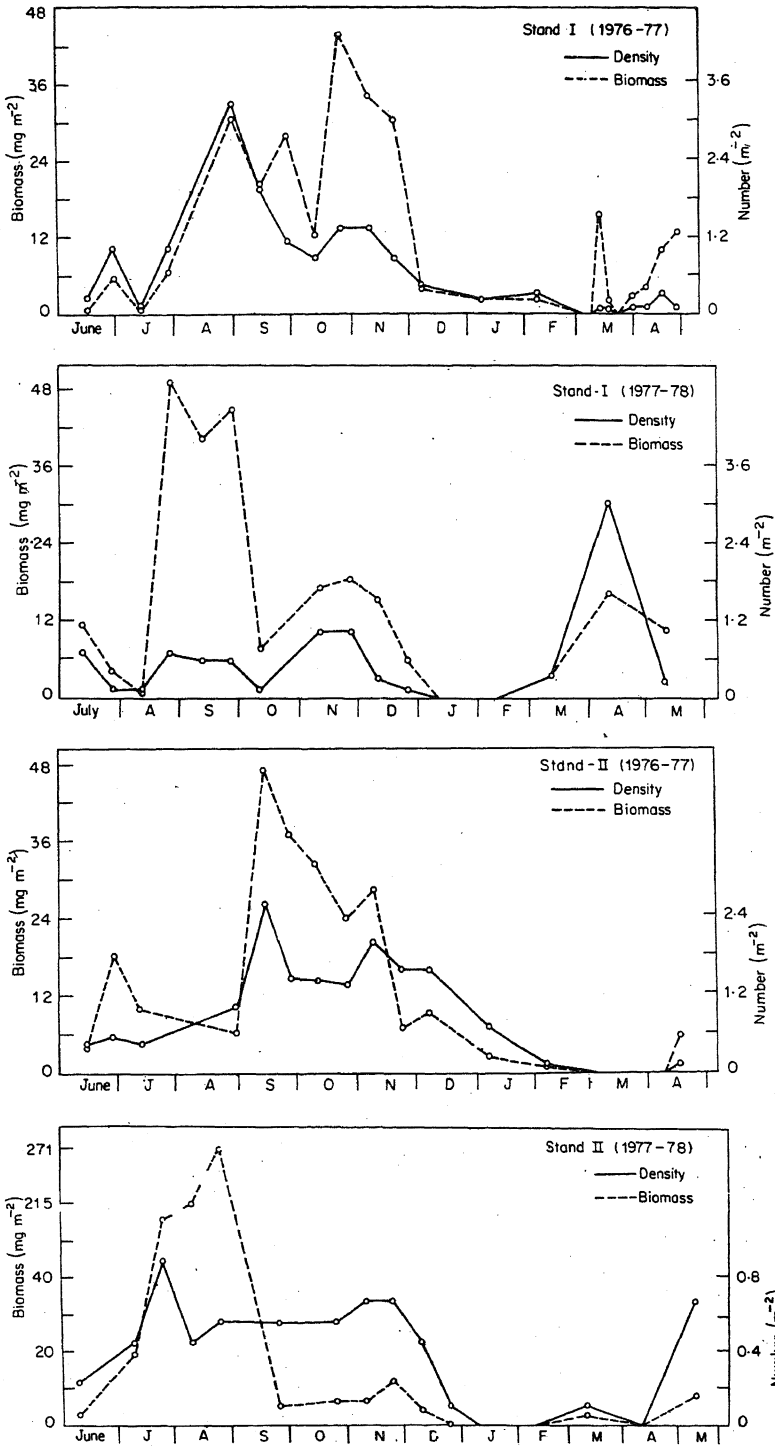
Maximum population density of order *Odonata* was 0.9 m^{-2} on stand I and 0.57 m^{-2} on stand II, whereas the maximum biomass values were 25.94 and 22.78 mg m^{-2} on stands I and II, respectively. *Agriidae* and *Libellulidae* were equally dominant in terms of density (0.68 m^{-2}) but *Libellulidae* alone was dominant with a maximum biomass of 25.94 mg m^{-2} , *I. senegalensis* and *R. nursei* had a maximum density of 0.68 m^{-2} , whereas *O. sabina* accounted for the maximum biomass.

Mantidae had a maximum population density of 1.02 and 1.14 m^{-2} and maximum biomass values were 33.61 and 267.11 mg m^{-2} on stands I and II respectively. *M. religiosa* was the most dominant species with a maximum density (1.02 m^{-2}) and a maximum biomass (267.11 mg m^{-2}).

Maximum population density for *Blattidae* was 0.23 and 0.45 m^{-2} , whereas maximum biomass was 5.02 and 10.54 mg m^{-2} on stands I and II respectively. *B. humbertiana* was dominant in terms of density (0.34 m^{-2}) and *Mareta* sp. had the maximum biomass (9.19 mg m^{-2}).

The maximum population density for *Neuroptera* on both the stands was 0.23 m^{-2} , whereas the maximum biomass values were 43.8 and 23.19 mg m^{-2} on stands I and II. A maximum density (0.23 m^{-2}) was recorded for both the families, while maximum biomass (43.8 mg m^{-2}) was recorded for Hemerobiidae. *P. paldus* and *H. dicax* were equally dominant with a density of 0.23 m^{-2} but *P. paldus* alone was dominant in terms of biomass (43.8 mg m^{-2}).

Maximum population density of *Diptera* was 2.74 and 1.98 m^{-2} and maximum biomass values were 8.6 and 13.47 mg m^{-2} on stands I and II respectively. Tabanidae with a density of 1.71 m^{-2} and Calliphoridae with a maximum biomass of 6.74 mg m^{-2} were the dominant families. *Tabanus* sp. had the maximum density (1.71 m^{-2}) whereas *Lucilla* sp. had the maximum biomass (6.74 mg m^{-2}).



Figures 1-4. Variation in population density and biomass of insects.

Table 2. Per cent contribution of various insect orders to total density and biomass (m^{-2}) during June 1976 to May 1978.

Taxa	Stand I		Stand II		Grassland as a whole	
	Density	Biomass	Density	Biomass	Density	Biomass
Orthoptera*	74.35	91.41	68.93	90.17	71.64	90.79
Homoptera*	4.04	1.06	0.85	0.18	2.45	0.62
Heteroptera*	1.94	1.06	3.65	1.64	2.79	1.35
Lepidoptera*	0.90	0.43	3.20	1.63	2.05	1.03
Hymenoptera*	10.49	1.80	14.23	1.52	12.36	1.66
Coleoptera*	2.50	0.82	2.66	0.70	2.58	0.76
Odonata	1.12	1.03	0.66	0.61	0.89	0.82
Mantoidea	1.27	1.39	1.84	2.14	1.56	1.76
Blattoidea	0.18	0.08	0.35	0.22	0.26	0.15
Neuroptera	0.17	0.25	0.17	0.42	0.17	0.34
Diptera	3.04	0.67	3.46	0.77	3.25	0.72

*These orders have been discussed separately elsewhere.

Table 3. Per cent contribution of different families to total density and biomass within an order during 1976-78.

Family	Stand I		Stand II		Grassland as a whole	
	Mean den.	Mean bio.	Mean den.	Mean bio.	Mean den.	Mean bio.
<i>Odonata</i>						
Coenagriidae	35.03	11.36	10.95	4.55	22.99	7.96
Agriidae	17.62	7.21	55.72	35.42	36.67	21.31
Libellulidae	47.35	81.43	33.33	60.03	40.34	71.73
<i>Neuroptera</i>						
Hemerobiidae	77.78	57.32	16.18	12.65	47.03	34.99
Myrmeliontidae	22.22	42.68	83.82	87.35	52.97	65.01
<i>Diptera</i>						
Tabanidae	22.70	25.39	6.89	9.98	14.80	17.68
Bombyliidae	—	—	0.96	1.47	0.48	0.74
Asilidae	2.72	4.26	1.92	5.74	2.32	5.00
Calliphoridae	14.47	6.94	24.85	20.10	19.66	13.52
Sarcophagidae	0.88	2.23	9.85	12.26	5.37	7.25
Tachinidae	43.00	36.48	29.82	30.51	36.41	33.49
Tripetidae	—	—	2.09	1.94	1.04	0.97
Muscidae	16.23	24.70	23.62	18.00	19.92	21.35

den. = density; bio. = biomass.

Moore (1953) recorded a density of 0.42 m^{-2} for Odonata, whereas Vats and Singh (1978) reported Libellulidae to be dominant in terms of density (0.33 m^{-2}) and biomass (26.62 mg m^{-2}). Lamotte (1975) reported a maximum density (0.71 m^{-2}) and biomass (84.06 mg m^{-2}) in a tropical savannah for Mantoidea. Data on population density and biomass of Blattidae and Neuroptera are lacking. Ricou (1967) reported dipterans with a density of 55 m^{-2} in the control plot but in the sown plot, it remained under 30 m^{-2} . Riegert *et al* (1974) recorded a mean density of 7.22 m^{-2} and a mean biomass of (56.18 mg m^{-2}) for Diptera in a natural untreated grassland.

Vats and Singh (1978) found Diptera to be insignificant in terms of density and biomass. In comparison, maximum density of Insecta in the present study was 3.3 m⁻² on a stand I and 2.63 m⁻² on stand II, whereas biomass values were 49.01 and 271.12 mg m⁻² on stands I and II respectively.

The absence of insects during summer and winter seasons was due to high temperature during summer (March-June) and low temperature during winter (October-February). Putnam (1962) and Davis and Gray (1966) pointed out that extremely high and low temperatures cause high mortality of insects. Vegetational cover influenced the number of species (Bhatnagar and Pfadt 1973). The absence of insects on some dates in the same month does not confirm their absence in that month but might have escaped collection.

Thus it can be concluded that type of vegetation and climatic factors played an important role in determining the number of species and population density of insects.

Acknowledgements

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Population ecology of *Schizodactylus monstrosus* (Drury) (Orthoptera) along the sand bed of Damodar river, West Bengal, India

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Abstract. The paper deals with the findings on some aspect of ecology of *Schizodactylus monstrosus* Drury. Studies on vertical distribution showed that the highest total density was in the 0 to 20 cm depth. Maximum concentration of the species was found in the first horizontal row. Monthly population structure on vertical distribution was also studied. Statistical analysis showed a significant correlation between the relative humidity and the distribution. Biomass of both the sexes and nymphal population was estimated.

Keywords. *Schizodactylus monstrosus*; sampling unit; vertical distribution; biomass.

1. Introduction

Schizodactylus monstrosus is a large, robustly built, ferocious looking burrowing nocturnal insect (Khattar 1972) and is commonly found in the sand bed of Damodar river. The morphology and anatomy of this peculiar orthopteran have been studied (Ragge 1957; Khattar 1965, 1972) as also its sub-social and other behaviour (Choudhuri and Bagh 1974). However the ecology of this insect has not been studied. The present paper reports the population dynamics in *S. monstrosus*, influence of its physical factors on the distribution pattern and estimation of biomass and its fluctuation in different months of the year (both adults and nymphs) in the sand bed of Damodar river.

2. Location and characteristics of the sampling sites

The Damodar river flowing along the south of Barsul village is located in the Burdwan District ($\sim 23^{\circ}$ - 24° N 87° - 88° E) 80 km west of Calcutta and 5 km south from Saktigarh Railway Station. The sand bed consists of light brown coarse sand, some sparsely distributed creepers and few patches of grass, the only vegetation on the sand bed.

3. Material and methods

3.1 Layout of the sampling sites

The stratified random sampling method with some modifications for sand bed was adopted (Yates and Finney 1942; Healy 1962; Abrahamsen 1969). The study area

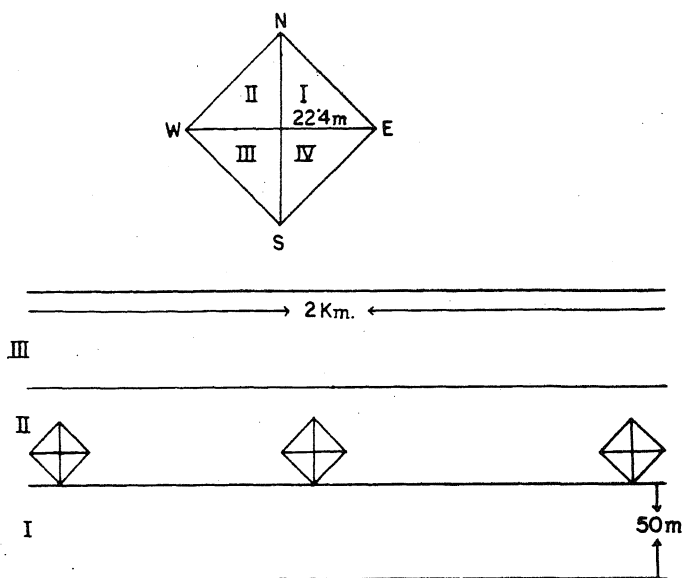


Figure 1. Layout of the sampling sites (each sample unit (NWSE) represents 1 ha).

extended for about 2 km from Barsul to Palla, the entire area of which was divided into three horizontal rows, parallel to each other, starting from the shore line to the bank of the river. The width of each horizontal row was 50 m and collections made from 162 sample units throughout the study period.

The area of the each sample unit was 0.1 ha and the sample unit was laid out on the sand bed with 22.4 m distances as half diagonals in all the four directions from the sampling unit with a nylon rope. For systematic data collection, the sample unit was divided into four quadrats (figure 1). The number of holes in each quadrat was counted from I and complete in quadrat IV *via* II and III. All the holes and the number of specimens collected from each burrow were serially numbered.

3.2 Collecting techniques

Collections were made in the morning at monthly intervals from June 1979 to November 1980. Nine units were collected per month *i.e.* at the rate of three randomly selected units from each row. The specimens were collected by digging both open and sand-plugged burrows in each sample unit. Separate sample unit was taken once a month in each row for vertical distribution study from June 1979 to May 1980. Relative humidity and temperature of the sand were recorded by a dial hygrometer and soil thermometer respectively. pH was determined by a digital pH meter.

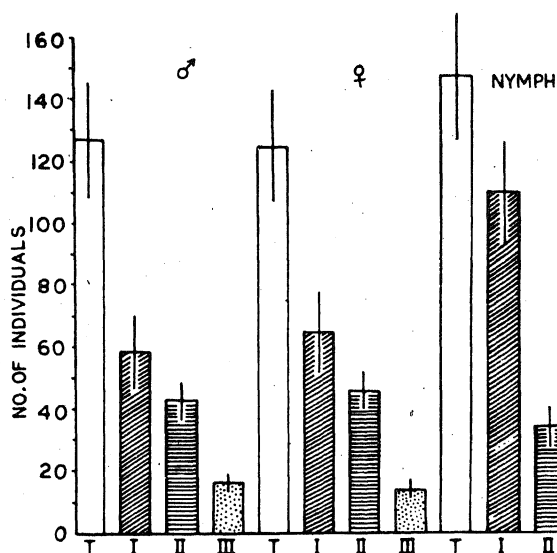
4. Observations

4.1 Horizontal distribution

A total of 7227 specimens were collected during the study of which 60.63% was made up by the first row whereas the third row comprised only 8.15%. Numerically

Table 1. Relative density of *S. monstrosus* in each row in the sand bed of Damodar river at Barsul area.

Rows Months	I			II			III		
	M	F	N	M	F	N	M	F	N
Jun. 1979	0.83	0.59	2.12	0.76	0.90	0.26	0.48	0.65	—
Jul.	1.13	1.08	2.10	0.62	0.80	0.21	0.51	0.32	0.01
Aug.	2.35	3.35	3.00	1.41	1.27	1.00	0.66	0.25	0.12
Sep.	2.21	2.10	3.49	0.72	0.72	0.69	0.18	0.37	0.07
Oct.	0.58	0.53	2.32	0.39	0.57	0.42	0.11	0.18	0.07
Nov.	0.29	0.44	1.00	0.64	0.62	0.37	0.08	0.14	0.06
Dec.	0.24	0.28	0.53	0.39	0.35	0.22	0.14	0.03	—
Jan. 1980	0.21	0.24	0.29	0.31	0.11	0.15	0.06	0.04	—
Feb.	0.71	0.62	0.32	0.42	0.46	0.24	0.14	0.06	—
Mar.	0.73	0.53	0.62	0.43	0.51	0.28	0.14	0.18	—
Apr.	0.66	0.58	0.66	0.35	0.30	0.04	0.12	0.14	—
May	0.82	0.83	0.72	0.28	0.42	0.48	0.14	0.17	—
Jun.	0.65	0.73	1.25	0.79	0.83	0.72	0.11	0.07	—
Jul.	1.34	1.04	2.28	0.94	1.13	0.24	0.54	0.15	0.03
Aug.	2.01	1.34	1.27	1.22	1.22	1.43	0.14	0.24	0.26
Sep.	1.59	1.26	2.46	0.42	0.62	1.08	0.32	0.26	0.14
Oct.	0.44	0.30	1.96	0.35	0.33	0.43	0.07	0.07	0.03
Nov.	0.30	0.35	0.94	0.25	0.29	0.36	0.11	0.10	—
Total	17.10	16.19	27.34	10.67	11.84	8.61	4.05	3.40	0.79

**Figure 2.** Mean of total male, female and nymph of *S. monstrosus* in three rows with standard error.

the nymphs represented the highest percentage of the total population recorded (36.76%) (table 1). Maximum number of nymphs were obtained from the first row (figure 2) and minimum from the third row.

The data obtained from monthly samples were plotted as monthly running means, together with the standard error of the mean (figure 3). The population of

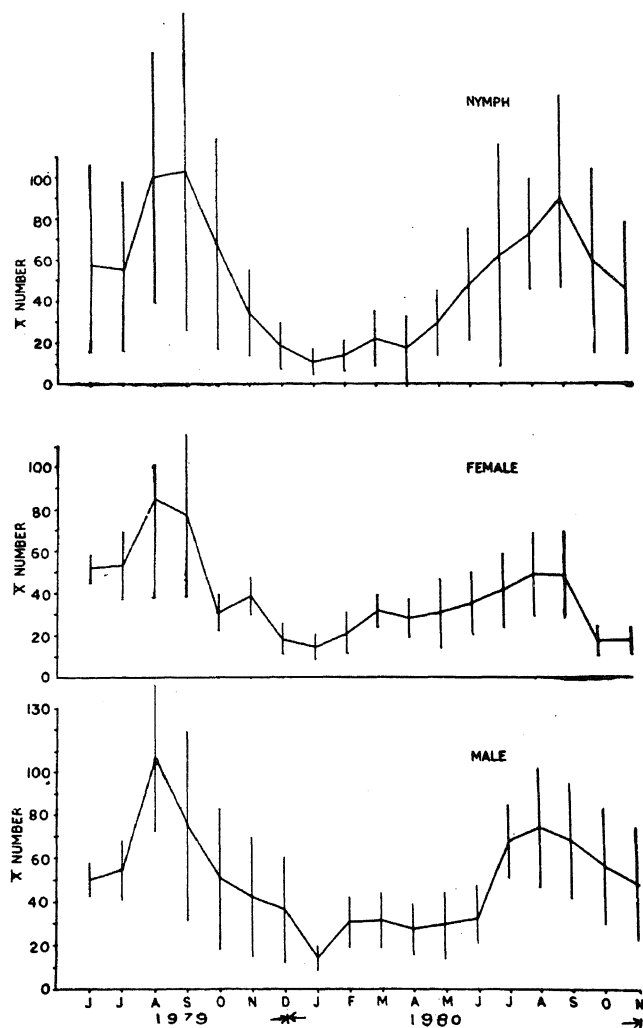


Figure 3. Month wise mean density of male, female and nymph population of *S. monstrosus*.

male and female reached its peak in August, while the nymphs showed its highest number in September in 1979 and 1980 (figure 3).

4.2 Vertical distribution

Monthly collections made for seasonal changes in vertical distribution of both adult and nymph populations are shown in figure 4. The highest total density of *Schizodactylus* occurred in the upper depth (0-20 cm) in the first and second row. The adults showed highest concentration in August in the two upper layers and in May maximum population occurred at 40-60 cm depth (figure 5), while the nymphal population showed its maximum concentration in September at 0-20 cm depth and in June at 20-40 cm depth, but the nymphs were absent at 40-60 cm depth (figure 6).

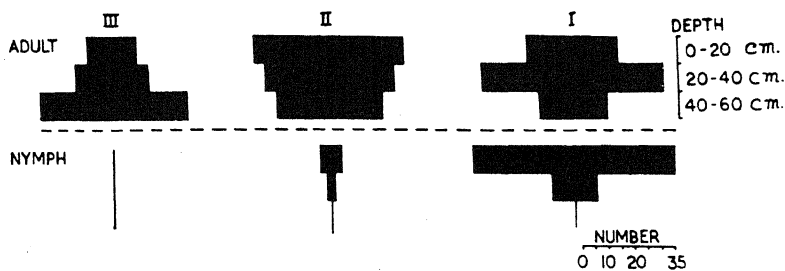


Figure 4. Vertical distribution of total population of *S. monstrosus* in each row.

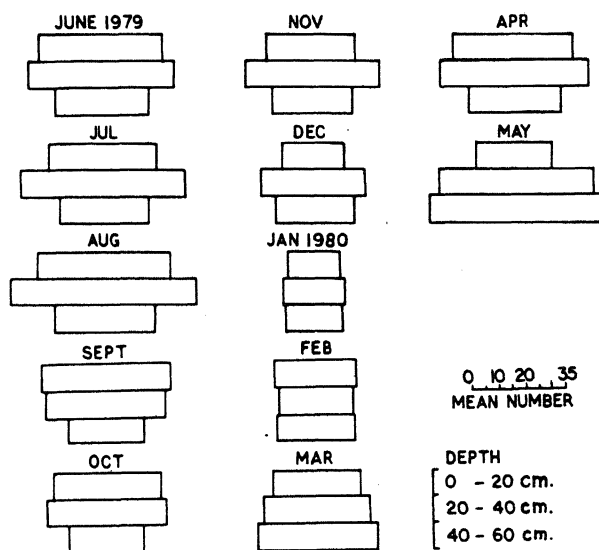


Figure 5. Month wise vertical distribution of adult *S. monstrosus* population.

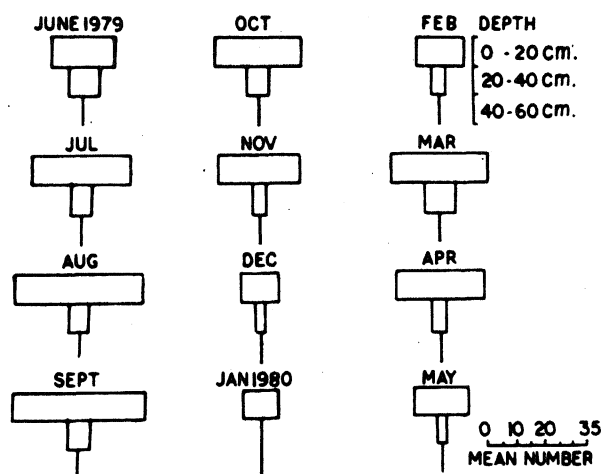


Figure 6. Month wise vertical distribution of *S. monstrosus* nymphal population.

4.3 Relation between physical factors

The pH was more or less same in all the three rows throughout the period of observation (figure 7). However, the RH and temperature fluctuated from one row to another and from one month to another. Relative humidity was maximum in August in all the rows and minimum in December in the first row and in May in second and third rows.

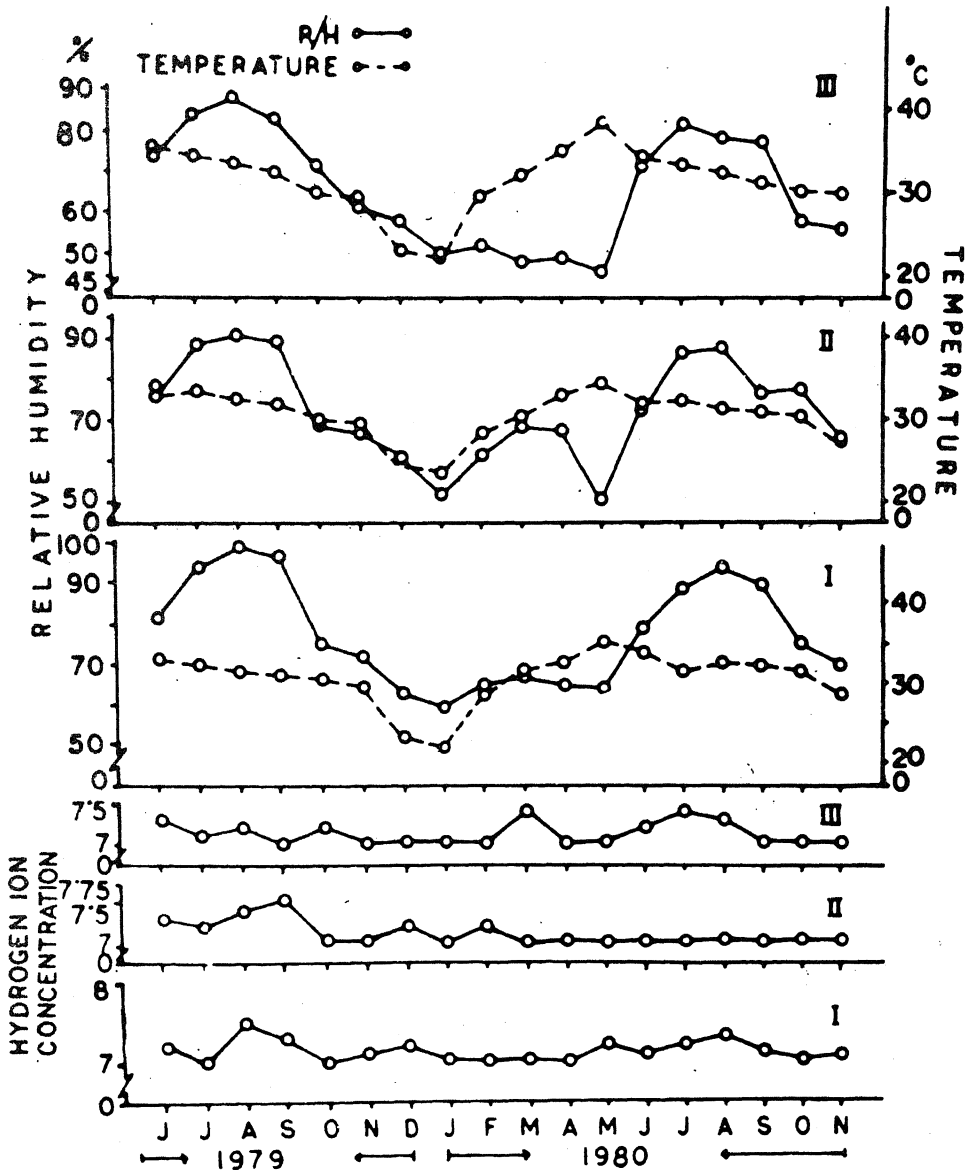


Figure 7. Monthly fluctuations of pH, R H and temperature in rows I, II and III.

4.4 Statistical analysis

To ascertain whether physical factors influenced the distribution of *Schizodactylus* and also to show the relationship between the population of one row with the others, the correlation coefficient and regression between the *Schizodactylus* and the physical factors under study were calculated (table 2, figure 8a-d). These results revealed high positive significant correlation (at 1% level) between the number of *Schizodactylus* and the relative humidity content of the sand in all the rows, while the other factors did not show any stable relationship.

Table 2. Relationship between *S. monstrosus* population and different parameters.

	Mean 'r' value	I row 'r' value	Regression $y = a + bx$
I row			
Population	232.33		
pH	7.13	0.74***	$7.04 + 0.00039x$
Temp	30.89	0.29*	$29.50 + 0.0068x$
RH	77.13	0.71***	$63.19 + 0.06x$
II row			
Population	123.39		
pH	7.10	0.34*	$7.03 + .00059x$
Temp	30.60	0.45**	$28.13 + 0.02x$
RH	71.37	0.97**	$47.93 + 0.19x$
III row			
Population	33.06		
pH	7.12	0.45**	$6.86 + .0079x$
Temp	31.64	0.30*	$29.99 + 0.05x$
RH	66.61	0.77***	$50.74 + 0.48x$
Population of I and II row	232.33	0.76***	$86.37 + 0.30x$
Population of I and III row		0.64***	$12.15 + 0.09x$
Population of II and III row	123.39	0.73***	$2.21 + 0.25x$

*** significant at 1% level; ** significant at 5% level; * non-significant.

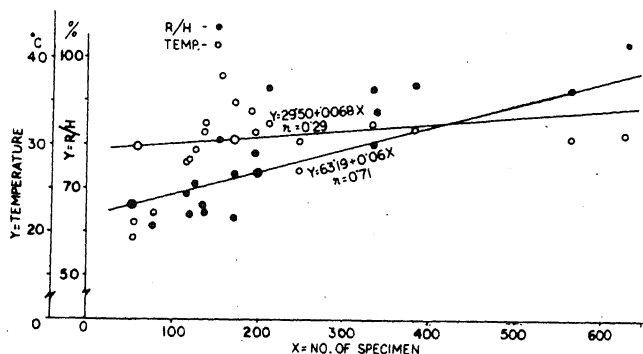


Figure 8. Regression of temperature and relative humidity on *S. monstrosus* population in first row.

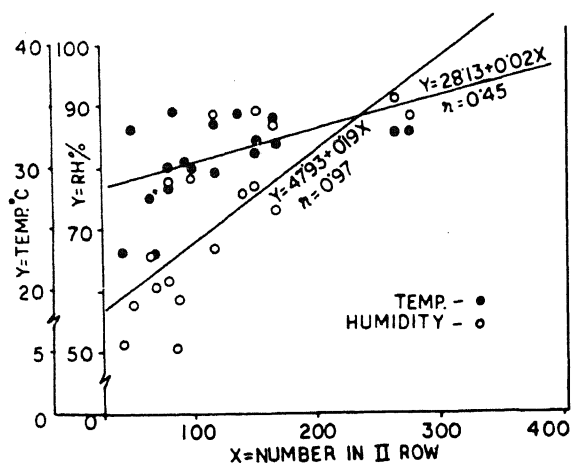


Figure 8a. Regression of temperature and relative humidity on *S. monstrosus* population in second row.

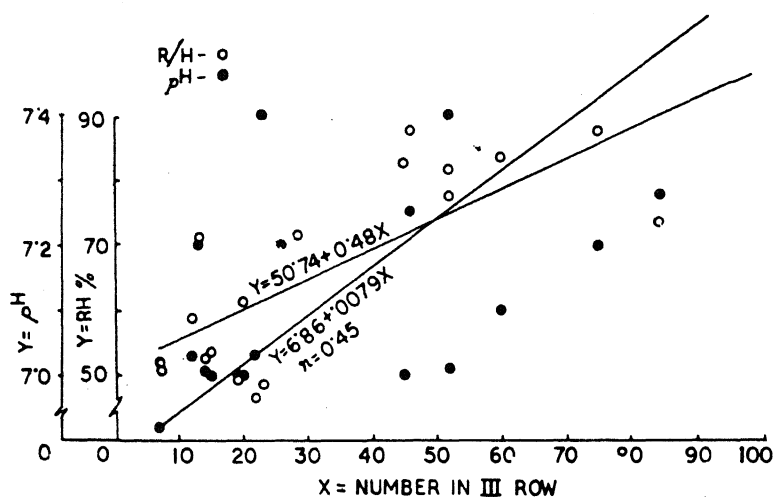


Figure 8b. Regression of pH and relative humidity on *S. monstrosus* population in second row.

4.5 Biomass of the population

Biomass is the most widely used measure of the populations (Hale 1966). The mean number of individuals and the biomass of both the sexes and nymphal population in each row per month in 0.1 hectare area are given in table 3 and figure 9. The female population comprised the highest percentage of biomass in all the three rows viz

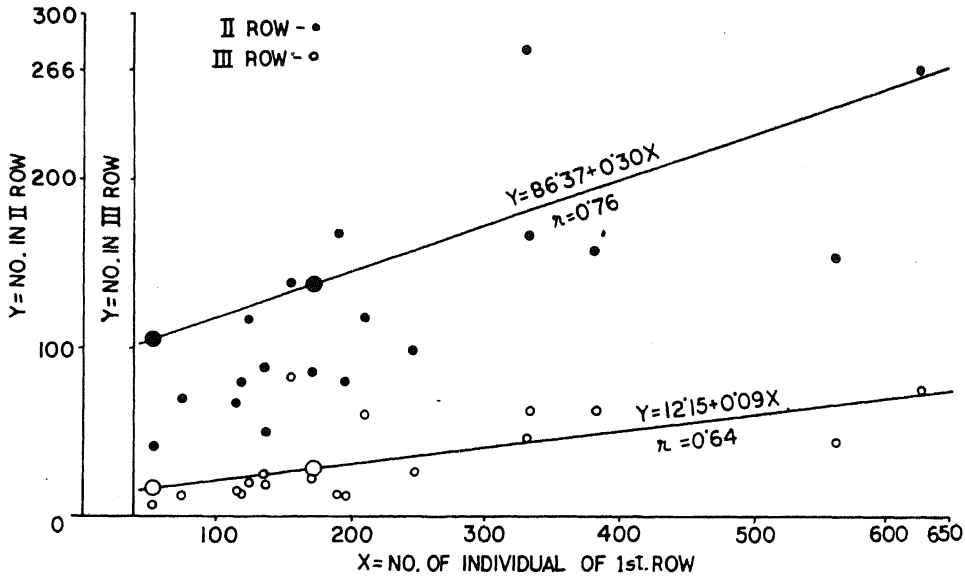


Figure 8c. Regression of second and third row *S. monstrosus* population on first row population.

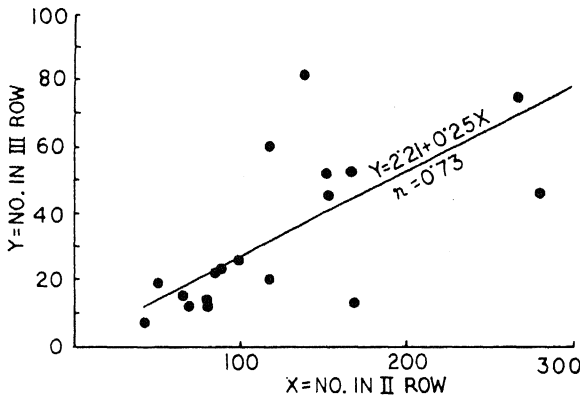


Figure 8d. Regression of third row *S. monstrosus* population on second row population.

27.15%, 19.17% and 5.74% in the 1st, 2nd and 3rd rows respectively. Contrary to this, the nymphal population contributes to the lowest percentage of the total biomass in the sand bed. The monthly fluctuation of biomass in each row was compared (figure 9a). It was seen that the first row yielded the maximum amount in each month, except in November 1979 and June 1980, when the second row showed highest amount. The biomass of male and female (average of three rows) in the studied area was highest in August and lowest in January, but the nymphal population showed maximum biomass during September (figure 10). From figure 2 it is evident that although the density of nymphal population was maximum in the 1st row the biomass added by this population was minimum (figure 10).

Table 3. Number (mean No./0.1 ha) and biomass (g dry wt/0.1 ha) of *S. monstrosus* at sand bed of Damodar river.

Months	I row					
	Male		Female		Nymph	
	Mean No.	Mean Biomass	Mean No.	Mean Biomass	Mean No.	Mean Biomass
Jun. '79	20.00	26.26	14.33	27.53	51.00	18.36
Jul.	27.33	35.88	26.00	49.95	50.67	18.24
Aug.	56.67	74.41	80.67	154.97	72.33	26.04
Sep.	53.33	70.02	50.67	97.34	84.00	30.24
Oct.	14.00	18.38	12.67	24.34	56.00	20.16
Nov.	7.00	9.19	10.67	20.50	24.00	8.64
Dec.	5.67	7.44	6.67	12.81	12.67	4.56
Jan.'80	5.00	6.57	5.67	10.99	7.00	2.52
Feb.	17.00	22.32	15.00	28.80	7.67	2.76
Mar.	17.67	23.20	10.67	20.50	15.00	5.40
Apr.	16.00	21.01	14.00	26.39	16.00	5.76
May	19.67	25.83	20.00	38.42	17.33	6.24
Jun.	15.67	20.57	17.67	33.94	30.00	10.80
Jul.	32.33	42.45	25.00	48.00	55.00	19.80
Aug.	48.33	63.46	32.33	62.11	30.67	11.34
Sep.	38.33	50.50	30.33	58.26	59.33	21.36
Oct.	10.67	14.01	7.33	14.08	47.33	17.00
Nov.	7.33	9.62	8.33	16.00	22.67	0.16
Total	412.00	541.12	390.00	749.19	658.87	237.19

Months	II row					
	Male		Female		Nymph	
	Mean No.	Mean Biomass	Mean No.	Mean Biomass	Mean No.	Mean Biomass
Jun. '79	18.33	24.07	21.67	41.63	6.33	2.28
Jul.	15.00	19.70	19.23	37.13	5.00	1.80
Aug.	34.00	44.64	30.67	58.92	24.00	8.64
Sep.	17.33	22.75	17.33	33.29	16.67	6.00
Oct.	9.33	12.25	13.67	26.26	10.00	3.60
Nov.	15.33	20.13	15.00	28.82	9.00	3.24
Dec.	9.33	12.25	5.33	16.00	5.33	1.92
Jan.'80	7.67	10.07	2.67	5.13	3.67	1.32
Feb.	10.00	13.13	11.00	21.13	5.67	2.04
Mar.	10.33	13.56	12.33	20.69	6.67	2.40
Apr.	8.83	10.94	7.33	14.08	1.00	0.36
May	6.67	8.76	10.00	19.21	11.67	4.20
Jun.	19.00	24.35	20.00	38.42	17.33	6.24
Jul.	22.67	29.77	27.33	52.50	5.67	2.04
Aug.	29.33	38.51	24.33	56.34	34.33	12.36
Sep.	10.00	13.13	15.00	28.82	26.00	9.36
Oct.	8.33	10.94	8.00	15.37	10.33	3.72
Nov.	6.00	7.88	7.00	13.45	8.67	3.12
Total	257.00	337.43	285.33	527.19	207.33	74.04

Table 3 (contd.)

Months	Male		III row Female		Nymph	
	Mean No.	Mean Biomass	Mean No.	Mean Biomass	Mean No.	Mean Biomass
Jun. '79	11.67	15.32	15.67	39.10	0	0
Jul.	12.33	16.19	7.67	14.73	0.33	0.12
Aug.	16.00	21.01	6.00	11.53	3.00	1.08
Sep.	4.33	5.69	9.00	17.29	1.67	0.60
Oct.	2.67	3.51	4.33	8.32	1.67	0.60
Nov.	2.00	2.63	3.33	6.40	1.33	0.48
Dec.	3.33	4.37	0.67	1.29	0	0
Jan. '80	1.33	1.75	1.00	1.92	0	0
Feb.	3.33	4.37	1.33	2.55	0	0
Mar.	3.33	4.37	4.33	8.32	0	0
Apr.	3.00	3.94	3.33	6.40	0	0
May	3.33	4.37	4.00	7.68	0	0
Jun.	2.67	3.51	1.67	3.21	0	0
Jul.	13.00	17.07	3.67	7.95	0.67	0.24
Aug.	3.33	4.37	5.67	10.89	6.33	2.28
Sep.	7.67	10.07	6.33	12.16	3.33	1.20
Oct.	1.67	2.19	1.67	3.21	0.67	0.24
Nov.	2.67	3.51	2.33	4.48	0	0
Total	97.67	128.24	82.00	158.43	19.00	6.84

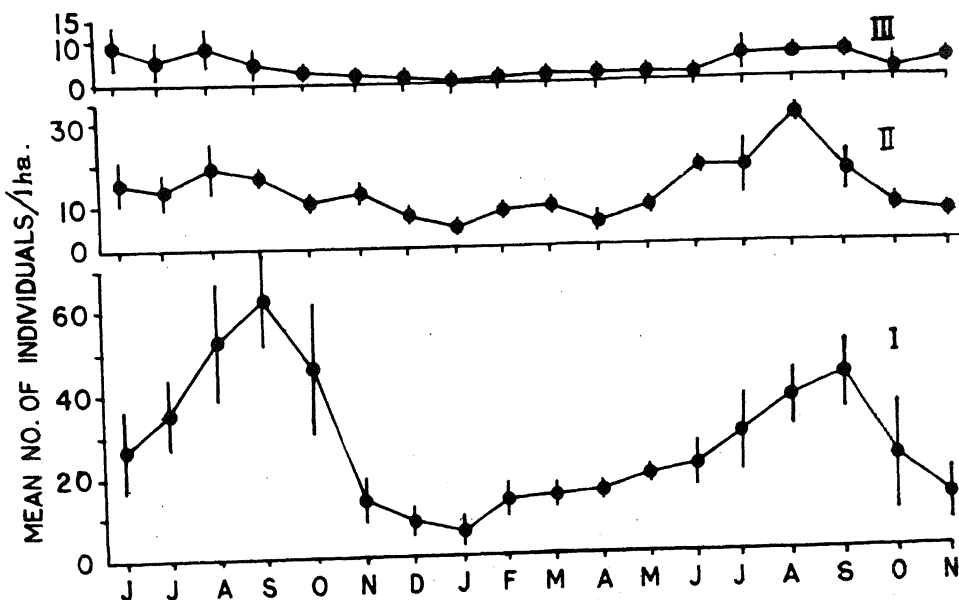


Figure 9. Row wise total population of *S. monstrosus*/month/0.1 ha. (vertical line indicates SE).

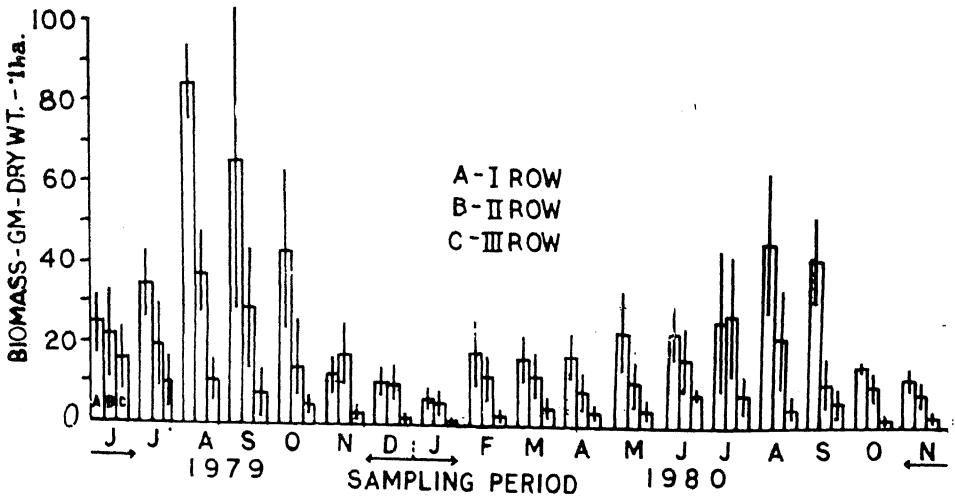


Figure 9a. Monthly changes in mean biomass in each row of *S. monstrosus* (vertical line indicates SE).

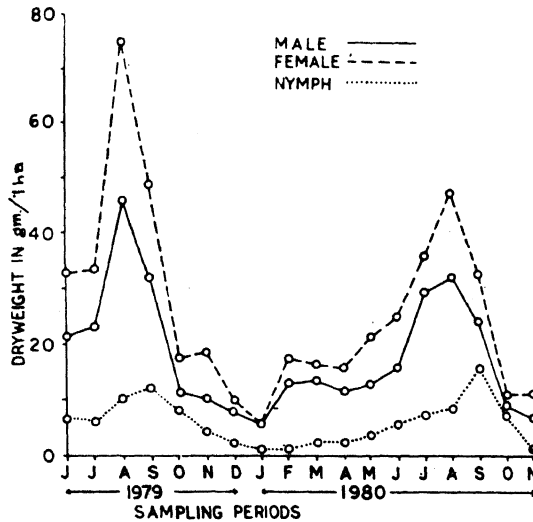


Figure 10. Monthly changes in biomass (g dry wt./0.1 ha.) of male, female and nymph population of *S. monstrosus* (mean of total biomass of 3 rows).

5. Discussion

Although the biology and ecology of different Orthoptera have been extensively studied, majority of the papers deal with Acrididae and Gryllidae, and Schizodactylidae received very little attention. Acrididae attain their maxima at some period between June and September (Khan and Aziz 1973). The present investigation shows that *Schizodactylus* populations attain its highest peak in

August and perhaps due to the cold spell, it was minimum in January. In the field all the nymphal stages are usually found during most of the sampling period, which indicates slow development of this insect from one instar to another and continuous generations. This may be due to the prevalence of major factors like moisture and food throughout the year in the sand bed. Similar observations were also made by Rentz and Weissman (1973) in silk spinning cricket *Cnemidettix* at California. According to Khattar (1972) the entire life cycle of *Schizodactylus* takes more than a year and it passes through nine instars. The highest density of *Schizodactylus* in the upper 0-20 cm depth in the first and second row may be due to the maximum nymphal population within this depth (figure 5). This observation agrees with similar studies on other typical soil inhabiting micro- as well as macroarthropods (Bellinger 1954; Poole 1961). The nymphal populations were completely absent from 40 to 60 cm depth which may be due to the absence of strong jaws during the nymphal stages as the tunnels are dug solely with the help of jaws (Carpentier 1953; Khattar 1972). Moreover, the nymphal density was higher in the front part of the first row which may be due to the presence of maximum moistened sand. The maximum number of adults in higher depth (40-60 cm) in the third row is due to the presence of less moisture content in the upper layer of this row as compared to other two front rows. As a result the adults go deep inside the sand to get favourable moisture. This confirmed the observation of Khattar (1972) who found a direct correlation between the depth of burrow and the sand conditions. The relative humidity of sand showed a direct correlation with the population density. The first row contained maximum population due to the maximum mean R/H concentration, contrary to this minimum population occurs from the third row, as it contained minimum mean R/H (figure 7). The influence of relative humidity was therefore much pronounced on the population fluctuation of *Schizodactylus*. It might be concluded that 31-32.5°C and 88.5-98.5% relative humidity in sand are the suitable combination for their peak population (figure 8, 8a). The density and biomass data on the burrowing orthopterans are not available. A maximum value of 75 mg/m² in the first row was found for female *Schizodactylus* in the present study. However, the grassland inhabiting the orthopterans were studied by several workers. Delvi and Pandian (1979) found 347 gcal/m² of *Poecilocerus pictus* near Bangalore field, Wiegert (1965) found 60 mg/m² for *Melanoplus* in Michigan according to Vanhook (1970). *Concephalus* had a maximum biomass of 200 mg per/m² and *Pteronemobius*, a field cricket, had a maximum biomass of 150 mg/m².

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***Meloidogyne* (Nematoda: Meloidogynidae) induced root galls of the banana plant *Musa paradisiaca*— A study of histopathology**

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Abstract. *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 was found to complete its life cycle within the roots of the banana plant *Musa paradisiaca* (cultivar Palayathodan). Egg sacs were confined entirely within the root. Second stage larvae penetrated the endodermis and on entering the stele succeeded in inducing multinucleate giant cells which were solely confined to the stele and never found in the cortex. Induction of giant cells and production of large number of females in banana roots showed that this is a susceptible host plant. Heavily infested roots showed histopathological changes which could lead to the blockage of conducting elements. This nematode is not very common in banana roots probably because second stage larvae emerging from the egg sacs reinfested the same roots and also because of the differential susceptibility of the various cultivars of the banana plant.

Keywords. *Meloidogyne incognita*; Nematoda; root histopathology; *Musa paradisiaca*; multinucleate giant cells; induction.

1. Introduction

The root-knot nematode *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 was reported from cultivars of *Musa paradisiaca*, an important crop plant in Kerala (Mohandas 1976). According to Blake (1972) this nematode is not a significant pest from the point of view of banana cultivation probably because the individual roots of the prolific adventitious root system of the banana plant is shortlived relative to the length of the life cycle of the nematode. Our preliminary studies were not in full agreement with the contention of the above author and hence the present work has been undertaken to provide information on aspects of the life cycle of *Meloidogyne incognita* within the root of *Musa* together with the histopathological changes brought about in the infected roots of the host.

2. Material and methods

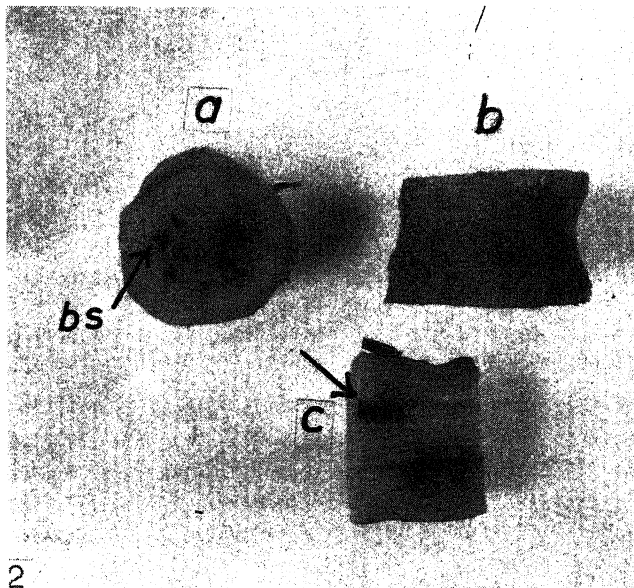
Thin hand sections of infected roots of *Musa paradisiaca* (cultivar Palayamthodan) fixed in FAA (formalin 6.5 ml; glacial acetic acid 2.5 ml; ethanol (50%) 100 ml) for 48 hr and preserved in 70% ethanol were used for histopathological studies. Nematodes *in situ* were stained using 0.1% acid fuchsin lactophenol (Goodey 1963). Dilute aqueous safranin gave good histological picture of the plant tissue. Nuclear and nucleolar conditions of giant cells were studied by staining sections using acetocarmine (saturated solution of carmine in 45% acetic acid).

3. Results and discussion

In sections of swollen roots (figure 1), thick, oval brown spots could be observed deep in the cortex and arranged radially around the stelar region just touching the vascular cylinder (figure 2 a,b). Each brown spot represented the position of an egg sac attached to the posterior end of the adult saccate female. Eggs at various stages of development and the second stage larvae hatched out from the eggs were seen in the egg sac. This means that all life stages of the nematode is found in the adventitious roots of the banana plant, a finding which is not in agreement with the contention of Blake (1972). Cortical parenchyma cells immediately surrounding the egg sacs became brown, thick-walled and opaque and formed a protective covering around the egg sac, except in the region where the latter was attached to the saccate female (figure 3). A similar condition was noted in the potato tubers (Finley 1981) except that in this case the protective covering completely surrounded the egg sac. It was suggested that the discolouration and the changes in the host cells might be due to the oxidation of phenolic substances and was induced by substances present in the gelatinous matrix of the egg sac. At the same time 5-7 layers of weakly stainable (by aqueous safranin), thin-walled and hypertrophied parenchyma cells in the cortex surrounded the brown area (figure 3), contributing to corresponding swellings (knots) formed in the root (figure 1). In *Musa* the egg sacs were never found protruding outside the root surface as has also been observed in the potato tubers (Finley 1981), contrary to the condition noted in most other host plants of *Meloidogyne* spp.

Normally the endodermis of *Musa* root was strongly developed with deposits in the cell wall which was thickened at the inner tangential side, except in the case of the passage cells which were distributed sparsely. Infective second stage larvae of the nematode penetrated this layer easily and entered the stele fully, where they started enlarging into saccate females. Endodermal cells adjacent to the region of infection, became highly enlarged and thin-walled (figure 4), and sometimes they showed a tendency to divide radially into two or more layers (figure 4). The larvae entered the stele presumably attracted by the phloem contents (Finley 1981) and induced the formation of large giant cells in the region of the pericycle, phloem and xylem parenchyma (figure 5). The number of giant cells observed at each site of infection varied, but not more than five radial cells were found in one group (figure 5). Taylor and Sasser (1978) and Arya (1980) suggested that giant cells appeared to be dependent on continual chemical stimulus from the feeding nematode. Giant cells were noticed only inside the stele and never in the cortex in *Musa* roots, as has been found by Krusberg and Nielsen (1958), Rao and Kumar (1973), Trivedi (1978) etc. But Balasubramonian and Rangaswami (1964) noted the presence of giant cells in the cortex also in sugarcane, while Chaturvedi and Khera (1979) found that giant cells occurred more frequently in the cortex of the roots of jute.

Giant cells had thick walls, dense granular cytoplasm and several nuclei (figure 6). Nuclei and nucleoli of giant cells showed hypertrophy. Some nuclei were irregularly spindle shaped or dumb-bell shaped (figure 6) about 24 μm long and 8.6 μm wide. The enlarged nuclei, nucleoli and dense granular cytoplasm in the giant cells indicated high metabolic activity as reported by Endo (1971). Bird (1974) suggested that the giant cells function as selective transporters over short distances



Figures 1-2. *Meloidogyne* infested roots of *Musa paradisiaca*. Arrows show regions of swelling. **2.** Sections of banana roots, **a.** TS through region of prominent swelling showing the position of mature females with egg mass, as brown spots (bs); **b.** Surface of the swollen root without any lesions; **c.** Longitudinal section through region of swelling, arrow indicating position of egg mass and mature female.

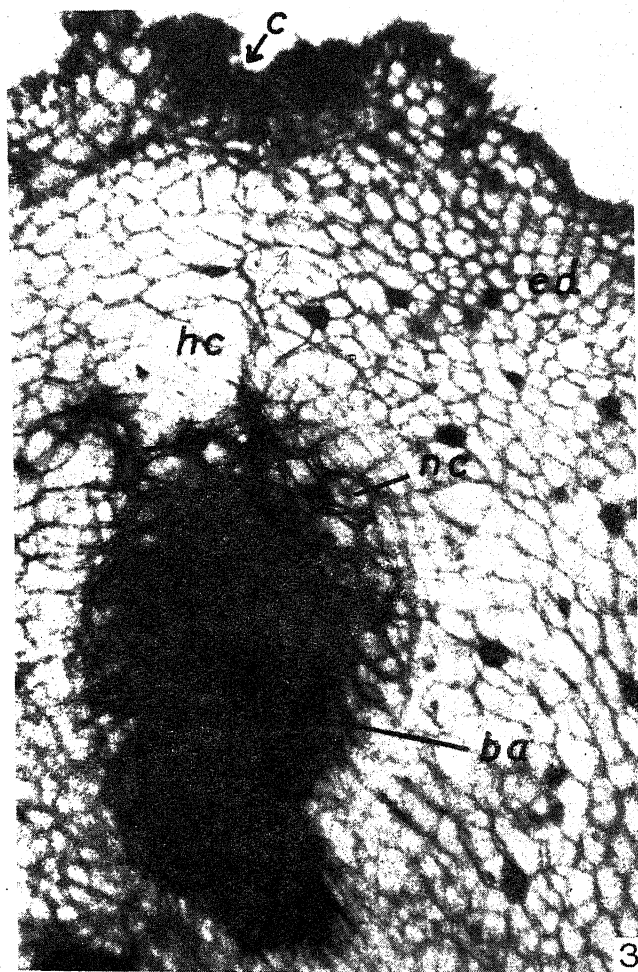
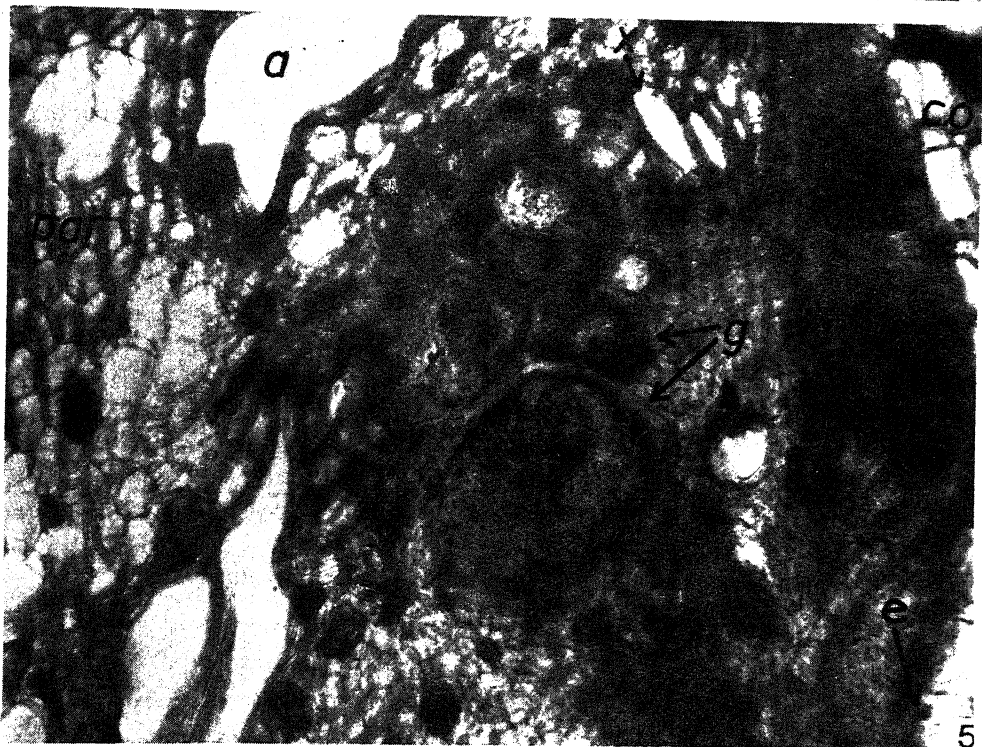
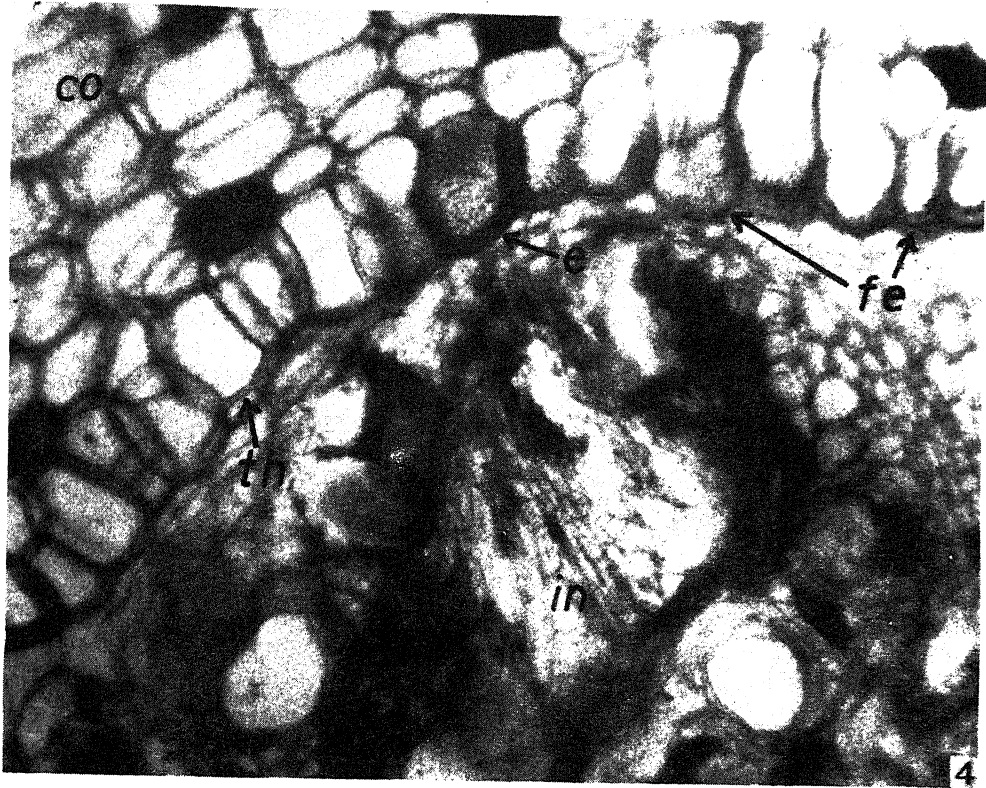


Figure 3. Transverse section of root stained in aqueous safranin (nc — cortical parenchyma cells which became brown, necrotic and thick walled; ba — brown host cells together forming a protective basket for the eggs sac; hc — hypertrophied cortical cells around the brown area; ed — exodermis; c — crack in the epidermis).

and that the developing larvae were entirely dependent on these cells for their nutritional requirements. The feeding nematode always had its head embedded in the stele adjacent to the giant cells. Endo (1971) suggested that those larvae which could induce large giant cells and derive sufficient nutrients became females, which later turned saccate with their body mostly pushed out from the stele in *Musa* roots. The formation of giant cells, developing of large number of females and production of several eggs by each female were considered as signs of high susceptibility of the host (Endo 1971; Sasser and Taylor 1978). In spite of the high susceptibility of the host roots the infestation of *Musa paradisiaca* by *Meloidogyne incognita* was not very common. The probable reason for this was apparently provided by a kind of re-infestation of the host roots noted in *Musa* in the course of the present study in which the second stage larvae emerging from the egg sacs in



Figures 4-5. (see captions on page 473.)

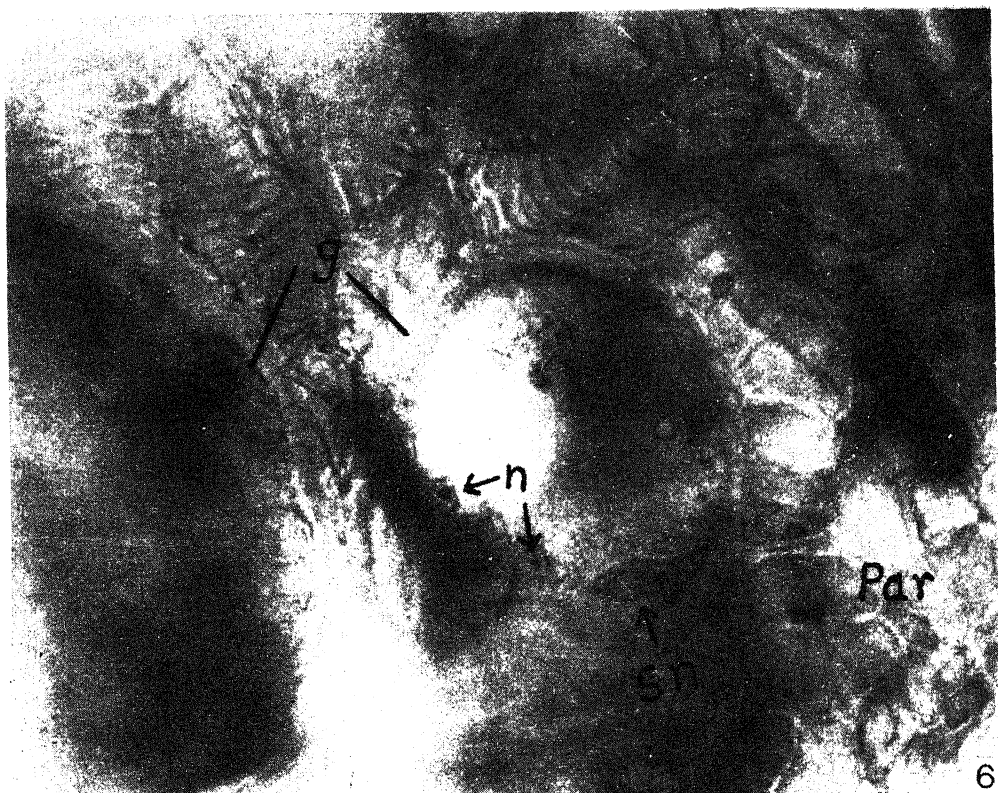


Figure 6. Transverse section of infected root stained in acetocarmine. (g — giant cells; n — nuclei; sn — spindle shaped nucleus; par — undifferentiated parenchyma cells within stele).

the cortex entered the stele of the same root. Further it is also probable that only certain cultivars of *Musa paradisiaca* are susceptible to the attack of *M. incognita* and 'Palayamthodan' is one such cultivar.

As a result of the enlargement of several females and also of many giant cells associated with the former, inside the stele, the phloem and xylem elements became displaced, malformed (figure 5) and at least partially blocked. Similar observations were made on other host plants by Trivedi (1978), Arya (1980), Rao and Kumar (1973) etc. Heavily infected banana plants showed such symptoms as poor growth, discolouration and yellowing of leaves etc., which have been reported on other host plants also (Singh 1973; Prasad 1972; Milne 1972; Shah and Raju 1977). From this it could be reasonably concluded that though the infestation of the banana plant by *Meloidogyne incognita* is not widespread in Kerala, the former is a susceptible host and that heavily infested plants showed severe phytopathological conditions of roots and external symptoms in the shoot system similar to those produced in other hosts heavily infested by this nematode.

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Figures 4 and 5. 4. TS through root stained in aqueous safranin showing modifications of endodermis. (co — cortex; e — normal endodermis cell; th — thin walled endodermis cell; fe — endodermis cells showing a tendency to divide radially; in — infected region within the stele). 5. Transverse section of the infected root stained in acetocarmine. (g — giant cells; co — cortex; e — endodermis; par — undifferentiated parenchyma cells in stele; x — dislocated xylem vessels; a — compressed 'trachea').

Food utilization in the fish *Channa striatus* exposed to sublethal concentrations of DDT and methyl parathion

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Abstract. Sublethal concentrations of DDT and methyl parathion (MP) in the medium significantly affected the rates of feeding, absorption and conversion in *Channa striatus*. Fish exposed to 250 ppb DDT or MP consumed 23 or 50% less food than those exposed to pesticide-free water. Correspondingly, absorption rate also decreased from 120 cal/g live fish/day in the control to 88 and 59 cal/g/day in those exposed to 250 ppb DDT and MP. Efficiency to convert the absorbed food into body substance dropped from 30% in the control to 6 and 12% in the 250 ppb DDT and MP groups. Metabolic rate of the control averaged to 84 cal/g/day; whereas exposure to DDT did not significantly affect the metabolic rate, a concentration of 250 ppb MP depressed the rate to 52 cal/g/day.

Keywords. Sublethal concentration; DDT; *Channa striatus*; food utilization; methyl parathion.

1. Introduction

Channa striatus is an obligate air-breathing fish, the juveniles of which thrive in overlapping zones of paddy fields, irrigation canals and ponds; hence the fish has been recommended for culture in the paddy fields (Iyengar 1962). Consequent to intensive agricultural operations, the fish is continuously exposed to pesticide stress. The shallowness of the habitat and the faster evaporation of water in the tropics concentrate the pesticide in the medium. Sublethal concentration of pesticide is known to alter the habitat, behaviour pattern, growth, reproductive potential and resistance to diseases through a variety of mechanisms in non-target species (Holden 1973). From elaborate static bioassay tests using *C. striatus*, the 96 hr LC_{50} values have been found to be 330 ppb for DDT and 880 ppb for MP (Bhaskaran 1980). Preliminary observations have revealed that *C. striatus* (15 g) survives over 40 days at sublethal concentration of 250 ppb of DDT or MP. Considerable information is available on the influence of exogenous factors such as temperature (Vivekanandan and Pandian 1977), ration (Vivekanandan 1976), PO_2 (Pandian *et al* 1977), and depth and volume of water (Pandian and Vivekanandan 1976) on the pattern of food utilization in some air-breathing fish. The present paper reports the influence of sublethal concentrations of DDT and MP on food utilization in *C. striatus*.

2. Material and methods

Specimens of *C. striatus* (15 ± 2 g) were acclimated separately for 7 days at chosen concentration (250, 125, 62 and 31 ppb) of DDT or MP in glass aquaria containing 5 l of water. The desired concentration of the pesticide in aquarium water was achieved by adding appropriate quantity of stock solution of the pesticide prepared in acetone. Technical grade DDT (Bharat Pulverisers, Bombay) and MP (Bayer, India) were used to prepare the stock solutions. The loach *Lepidocephalichthys thermalis* served as prey during acclimation and experiment.

Healthy acclimated *C. striatus* were weighed and used for the feeding experiment, which lasted for 22 days. Two series (DDT and MP) of experiments, each consisting of 3 to 6 individuals for each test concentration were conducted. A control group was also fed separately exposing the fish to pesticide-free water. All the fish were fed *ad libitum* on weighed quantities of live *L. thermalis* once a day. The residual prey left in the aquarium were collected every day and dried. Faeces egested by the fish was collected by filtering the entire aquarium water once in 4 days and drying the residue. After removing the water for faeces collection, the aquarium was filled with fresh water and the pesticide concentration was maintained by adding appropriate quantity from the stock solution. At the end of the experiment the fish was weighed and dried. Water and caloric contents of a few sample fish were estimated and these were used to determine the values for the test fish (see also Maynard and Loosli 1962). The food, faeces and fish were dried to weight constancy at 90°C and weighed to an accuracy of 0.1 mg. Caloric content was determined in Parr 1411 semi-micro bomb calorimeter following the standard procedure.

The scheme of energy balance followed in the present study was that of IBP formula of Petruszewicz and MacFadyen (1970), usually represented as: $C = F + U + M + P$, where C is the food consumed, F the faeces, U the urine, M the energy lost as heat due to metabolism and P the growth (= conversion). Food energy absorbed (A) was calculated by subtracting F from C , and P was calculated by subtracting the energy content of the fish at the commencement of the experiment from that at the termination. M was estimated by subtracting P from A ; the M value thus arrived at includes a constant (see Nirmala 1981) but negligible fraction of U (Winberg 1956). Rates of feeding, absorption and conversion and metabolism were calculated by dividing the respective quantum of energy by the products of fish weight (g) and duration of the experiment (22 days) and expressed in terms of cal/g live fish/day. Efficiency of absorption was calculated by relating A to C and conversion efficiency (K_p) relating P to A .

3. Results

Exposure to sublethal concentration of the pesticides significantly influenced the caloric content of the fish and faeces (table 1). With increasing concentration of the pesticides in the medium, caloric content of the fish decreased; the decrease was from 5389 cal/g dry weight in the control to 4671 or 4725 cal/g in the fish exposed to 250 ppb DDT or MP. Depletion of energy per unit weight of organismic matter is considered to reflect utilization of high energy containing lipids for

Table 1. Caloric content of *C. striatus* and faeces as functions of DDT and MP concentrations.

Pesticide	Exposure concentration (ppb)	Calorific content (cal/g dry weight)	
		Fish	Faeces
DDT	Control	5389 ± 226	1333 ± 42†*
	31	5189 ± 217	1440 ± 98
	62	4989 ± 347	1548 ± 75
	125	4830 ± 315	1645 ± 69
	250	4671 ± 261	1745 ± 86*
MP	31	5123 ± 289	1405 ± 192
	62	4857 ± 217	2475 ± 139
	125	4791 ± 159	1548 ± 141
	250	4725 ± 165	1612 ± 121†

**t* = 6.088 *P* < 0.01; †*t* = 3.080 *P* < 0.05

metabolism (see Pandian 1983); it is likely that *C. striatus* under the pesticide stress mobilize fat reserves to meet the higher metabolic energy demand. On the other hand, energy content of faeces increased from 1333 cal/g in the control to 1745 or 1612 cal/g in those exposed to 250 ppb DDT or MP (table 1), suggesting that a fraction of the prey containing energy-rich lipid is not digested and absorbed.

The feeding rate of the control fish averaged, 125 cal/g/day (table 2). In the fish exposed to 31, 62 and 125 ppb of DDT, the feeding rate was not significantly different from those exposed to the corresponding concentrations of MP. But at 250 ppb of DDT or MP, the rate was only 96 to 63 cal/g/day. At this sublethal concentration, it decreased to about 50% of the control value in the DDT and MP series, respectively; the feeding capacity of *C. striatus* is more severely impaired by MP than DDT.

Trends obtained for absorption rate of the fish in different groups of DDT and MP series were more or less similar to the respective values obtained for feeding rate (figure 1). Fish exposed to 250 ppb DDT or MP absorbed 88 or 59 cal/g/day compared to 120 cal/g/day absorbed by that in the control group (table 2). Absorption efficiency, which was around 94% was not affected by the exposure to the pesticides; however, statistical comparison of the mean efficiency values of the control fish and those exposed to the highest concentrations of DDT or MP showed that efficiency was affected at the highest tested sublethal concentration of the pesticides.

Exposure to the pesticides significantly affected the rate and efficiency of food conversion. The control fish converted at the rate of 36 cal/g/day compared with 6 and 7 cal/g/day in the highest concentration of DDT and MP, respectively. Conversion efficiency also decreased to 6 and 12% in these groups.

With increasing pesticide concentration and stress, *C. striatus* channelled a greater proportion of the absorbed food energy into metabolism. Metabolic rate of the fish in the DDT series oscillated around 85 cal/g/day (table 2). In the MP series, it progressively decreased from 83 cal/g/day in the 31 ppb group to 52 cal/g/day in the fish exposed to the highest concentration.

Table 2. Food utilization in *C. striatus* (15 g) exposed to different concentrations of DDT and MP.

Parameter	Control	Concentration (ppb)							
		31		62		125		250	
		DDT	MP	DDT	MP	DDT	MP	DDT	MP
Feeding rate	125±7.1	117±6.2	116±6.2	109±2.7	103±6.6	90±4.6	84±6.2	96±8.2	63±6.4
Absorption rate	120±6.8	111±5.9	110±5.8	103±2.3	98±6.2	83±4.3	80±4.3	88±7.5	59±6.0
Conversion rate	36±3.2	24±3.1	27±5.9	17±4.4	20±2.9	10±1.6	14±1.7	6±1.6	7±1.0
Metabolic rate	84±4.1	87±2.9	83±6.7	86±2.8	78±5.4	73±3.3	66±7.5	82±6.3	52±6.0
Absorption efficiency	96±0.7	95±1.2	96±1.3	94±1.7	95±2.0	93±2.0	5±1.0	92±1.9	93±1.5
Conversion efficiency	30±1.3	21±1.5	24±5.1	18±1.9	19±2.7	11±1.5	18±3.2	6±1.5	12±1.8

Each value represents the mean (±SD) performance of 3-6 individuals fed *ad libitum* on live *Lepidocephalichthys thermalis* for a period of 22 days at 29°C. The rates are expressed in cal/g/day and the efficiencies in %.

Student's *t* test: Feeding rate : 31 ppb DDT vs 31 ppb M.P. *t* = 0.197; *P* > 0.5
 62 ppb DDT vs 62 ppb M.P. *t* = 1.457; *P* > 0.5
 125 ppb DDT vs 125 ppb M.P. *t* = 1.346; *P* > 0.5

Absorption efficiency : 250 ppb DDT vs Control *t* = 2.79; *P* < 0.05
 250 ppb M.P. vs Control *t* = 2.60; *P* ≤ 0.05

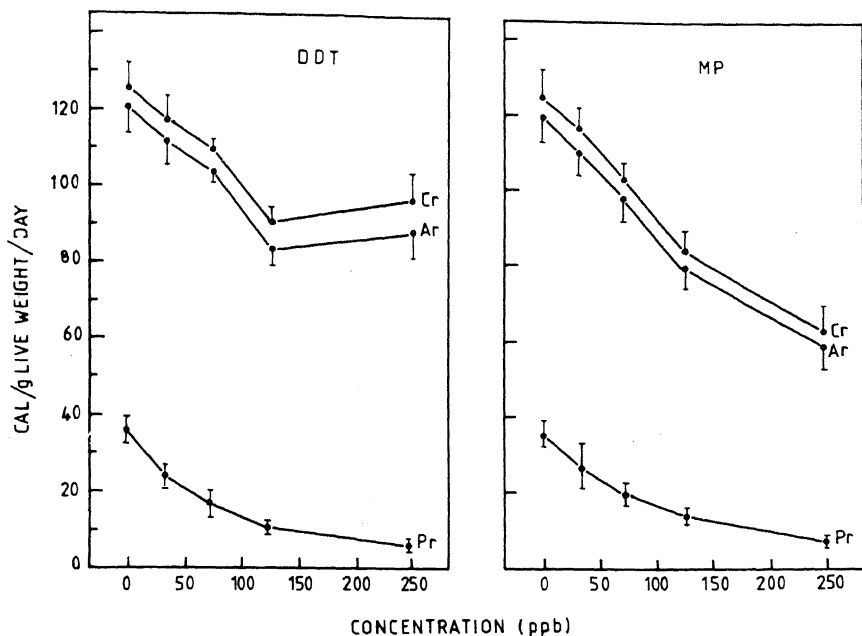


Figure 1. Effect of sublethal concentrations of DDT (left panel) and MP (right panel) on consumption (Cr), absorption rate (Ar) and conversion rate (Pr) in the fish *Channa striatus*. Each value represents the mean (vertical line = SD) performance of 3 to 6 individuals fed for 22 days.

4. Discussion

Despite *ad libitum* supply of live prey, sublethal concentrations of DDT and MP significantly affected feeding and metabolism, and hence growth of *C. striatus*. Exposure to sublethal concentrations of MP depressed feeding rate to 50% and metabolism to 60% of the respective control values, whereas that of DDT reduced feeding (to 80%) but not metabolism. The observed decrease in conversion efficiency of the fish may be attributed mainly to the depressing effect of MP on feeding, and to increased metabolism in that exposed to DDT. Webb and Brett (1973) observed significant decreases in rate and efficiency of conversion in *Oncorhynchus nerka* exposed to sublethal concentration of the organochlorine pesticide, sodium pentachlorophenate. They attributed the depressing effect of the pesticide on feeding as solely responsible for the decreased rate and efficiency of food conversion. The present study indicates that (i) reduction in feeding (as is the case with MP) and/or (ii) the need for allocation of a greater proportion of the absorbed food energy for metabolism (as is the case with DDT) are responsible for the negative effects of the pesticides on food conversion.

Decrease in feeding rate of the fish exposed to MP appears to be due to the impairment of impulse transmission (O'Brien 1976) and physical deformation (Bengtsson 1975) leading to depressed swimming activity, predatory ability and feeding capacity. The report of Bhaskaran (1980) that among *C. striatus* exposed to 96 hr LC_{50} of MP, about 11% suffered from physical deformations (see also McCann and Jasper 1972 for vertebral deformations in *Lepomis macrochirus*

exposed to MP) supports the above conclusion. In natural habitat, sublethal concentrations of MP may let *C. striatus* survive, but with poor predatory efficiency.

Metabolic rate of fish depends to a considerable extent on feeding rate (e.g. Pandian and Vivekanandan 1976; Vivekanandan and Pandian 1977). Metabolic rate of *C. striatus* exposed to MP decreased with feeding rate, while those exposed to DDT maintained the rate at a level more or less on par with that of the control fish, despite the decrease in feeding rate. Bhaskaran (1980) observed that by uncoupling oxidative phosphorylation from the respiratory chain, DDT decreases the respiratory control (RC) ratio and increases O_2 uptake overrides the depression in metabolic rate owing to the decrease in the feeding rate and as a result, the metabolic rate of the fish under DDT stress is more or less equal to that of the control.

Decrease in the absorption efficiency of *C. striatus* in the 250 ppb DDT or MP series is understandably due to the increase in the calorific content of faeces over that of the control (table 1). The suggestion of Miller and Kinter (1977) that by altering protein conformation in cell membranes, DDT affects activity of enzymes such as ATPase which aid in the absorption of nutrients is worth considering in this content (see also Bhaskaran 1980).

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